Załącznik 3

AUTOREFERAT
DESCRIPTION OF THE SCIENTIFIC ACHIEVEMENTS

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TABLE OF CONTENTS

I. NAME and SURNAME: Magdalena Sowa-Kućma

II. DIPLOMAS, DEGREES

2008 – Ph.D. in Medical Sciences, in the field of medical biology – Institute of Pharmacology Polish Academy of Sciences, Department of Neurobiology

Thesis entitled: „The role of zinc in the adaptive mechanisms after antidepressant drugs"

Supervisor: *prof. dr hab. Gabriel Nowak*

2003 – M.Sc. in Biology – Jagiellonian University, Faculty of Biology and Earth Sciences, Department of Animal Physiology

Thesis entitled: „Effect of PCB126 (3,3',4,4',5-pentachlorobiphenyl) and PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl) on steroidogenesis and apoptotic processes of cells collected from ovarian follicles of slaughter pigs"

Supervisor: *prof. dr hab. Ewa Gregoraszczuk*

2003 – Teacher Training Center, Jagiellonian University, Krakow, Poland – teacher's diploma

III. INFORMATION ON HITHERTO EMPLOYMENT IN SCIENTIFIC INSTITUTIONS

A. Employment in scientific institutions

Oct 2017 – present University of Rzeszow, Medical Faculty, Institute of Clinical and Experimental Medicine, Department of Human Physiology and Pathophysiology **(**Assistant Professor)

Nov 2009 –Oct 2015 University of Rzeszow, Faculty of Biotechnology, Department of Animal Physiology and Reproduction (Assistant Professor)

Jul 2008 – Sep 2018 Institute of Pharmacology Polish Academy of Sciences, Krakow, Department of Neurobiology/ Department of Behavioral Neuroscience and Drug Development (Assistant)

Dec 2007 - Jun 2008 Institute of Pharmacology Polish Academy of Sciences, Krakow, Department of Neurobiology (Technical Assistant)

Dec 2003 - Nov 2007 Institute of Pharmacology Polish Academy of Sciences, Krakow, Department of Neurobiology – Ph. D. student

B. Appointment to management positions

Oct 2017 – Head of the Department of Human Physiology, Institute of Experimental and Clinical Medicine, Medical Faculty, University of Rzeszów

Aug 2017 – Head of the Laboratory of Innovative Research of Cardiovascular and Respiratory Systems, Centre for Innovative Research in Medical and Natural Sciences, University of Rzeszów

IV. SCIENTIFIC ACHIEVEMENT, ACCORDING TO ART. 16 SEC. 2 OF THE BILL FROM 14 MARCH 2003 R. ON SCIENTIFIC DEGREES AND TITLE AND DEGREES AND TITLE IN THE DOMAIN OF ARTS (J. LAWS OF 2016, ITEM 882, AS AMENDED IN J. LAWS OF 2016, ITEM 1311)

A. Title of the scientific achievement:

"Zinc, NMDA receptors, oxidative stress, inflammation and their relationships in the pathophysiology of depression"

The obtained scientific achievements which form the **basis of the habilitation thesis** have been presented in a monothematic series of **8 scientific papers (7 original and 1 review papers), published in the period 2013-2018.** The **total impact factor (IF)** of these publications is: **24.593;** the total KBN/MNiSW score is: 230.

B. Author/authors, title/titles of publication, year of publication, publisher

(*The publications are presented in the order of their discussion in section IVC*)

1. **Sowa-Kućma M** (*corresponding author*), Szewczyk B, Sadlik K, Piekoszewski W, Trela F, Opoka W, Poleszak E, Pilc A, Nowak G.: Zinc, magnesium and NMDA receptor alterations in the hippocampus of suicide victims.
J Affect Disord. 2013; 151(3):924-31. doi: 10.1016/j.jad.2013.08.009
IF: 3.705 KBN/MNiSW: 35 Publisher: Elsevier
My contribution to this work concerned taking part in the plannig and setting up of the study, implementation of the whole biochemical analyzes as well as the analysis and interpretation of the obtained results, reviewing the literature, writing the manuscript, formulating answers for the reviewers.
*I estimate my contribution to be **80%.***

2. Siwek M, **Sowa-Kućma M** (*first co-author; corresponding author*), Dudek D, Styczeń K, Szewczyk B, Kotarska K, Misztakk P, Pilc A, Wolak M, Nowak G. Oxidative stress markers in affective disorders. **Pharmacol Rep. 2013; 65(6):1558-71.**
IF 2.165 KBN/MNiSW: 25 Publisher: Elsevier
My contribution to this work concerned taking part in the reviewing the literature, writing the manuscript and formulating answers for the reviewers.
*I estimate my contribution to be **40%.***

3. Sowa-Kućma M (*corresponding author*)**,** Styczeń K, Siwek M, Misztak P, Nowak RJ, Dudek D, Rybakowski JK, Nowak G, Maes M.: Lipid Peroxidation and Immune Biomarkers Are Associated with Major Depression and Its Phenotypes, Including Treatment-Resistant Depression and Melancholia.
Neurotox Res. 2018; 33(2):448-460. doi: 10.1007/s12640-017-9835-5
IF: 2.942 KBN/MNiSW: 25 Publisher: Springer
My contribution to this work concerned taking part in the plannig and setting up of the study, the running of the study (preparation and collection of blood samples, development and optimization of the biochemical measurements; implementation of the whole biochemical analyzes), creating the database as well as statistical analysis and interpretation of the obtained results, reviewing the literature, writing the manuscript, formulating answers for the reviewers.
*I estimate my contribution to be **75%.***

4. Styczeń K, **Sowa-Kućma M** (*corresponding author*), Siwek M, Dudek D, Reczyński W, Szewczyk B, Misztak P, Topór-Mądry R, Opoka W, Nowak G.: The serum zinc concentration as a potential biological marker in patients with major depressive disorder.
Metab Brain Dis. 2017; 32(1):97-103. doi: 10.1007/s11011-016-9888-9
IF: 2.297 KBN/MNiSW: 25 Publisher: Springer
My contribution to this work concerned taking part in the plannig and setting up of the study, the running of the study (preparation and collection of blood samples), creating a database as well as the analysis and interpretation of the obtained results, reviewing the literature, writing the manuscript, formulating answers for the reviewers.
*I estimate my contribution to be **40%.***

5. Siwek M, **Sowa-Kućma M**, Styczeń K, Szewczyk B, Reczyński W, Misztak P, Topór-Mądry R, Nowak G, Dudek D, Rybakowski JK.: Decreased serum zinc concentration during depressive episode in patients with bipolar disorder.
J Affect Disord. 2016; 190:272-277. doi: 10.1016/j.jad.2015.10.026.
IF: 3.570 KBN/MNiSW: 35 Publisher: Elsevier
My contribution to this work concerned taking part in the plannig and setting up of the study, the running of the study (preparation and collection of blood samples), creating the database as well as the statistical analysis and interpretation of the obtained results, reviewing the literature, writing the manuscript, formulating answers for the reviewers.
*I estimate my contribution to be **40%.***

6. **Sowa-Kućma M** (*corresponding author*)**,** Styczeń K, Siwek M, Misztak P, Nowak RJ, Dudek D, Rybakowski JK, Nowak G, Maes M.: Are there differences in lipid peroxidation and immune biomarkers between major depression and bipolar disorder: Effects of melancholia, atypical depression, severity of illness, episode number, suicidal ideation and prior suicide attempts.
Prog Neuropsychopharmacol Biol Psychiatry. 2018; 81:372-383. doi: 10.1016/j.pnpbp.2017.08.024
IF: 4.187 KBN/MNiSW: 35 Publisher: Elsevier
My contribution to this work concerned taking part in the plannig and setting up of the study, the running of the study (preparation and collection of blood samples, development and optimization of the biochemical measurements; implementation of the whole biochemical analyzes), creating the database as well as analysis and interpretation of the obtained results, reviewing the literature, writing the manuscript, formulating answers for the reviewers.
*I estimate my contribution to be **75%.***

7. Doboszewska U, Szewczyk B, **Sowa-Kućma M**, Noworyta-Sokołowska K, Misztak P, Gołębiowska J, Młyniec K, Ostachowicz B, Krośniak M, Wojtanowska-Krośniak A, Gołembiowska K, Lankosz M, Piekoszewski W, Nowak G.: Alterations of Bio-elements, Oxidative, and Inflammatory Status in the Zinc Deficiency Model in Rats.
Neurotox Res. 2016; 29(1):143-54. doi: 10.1007/s12640-015-9571-7
IF: 3.140 KBN/MNiSW: 25 Publisher: Springer
My contribution to this work concerned taking part in the plannig and setting up of the study, the running of the study (development, optimization and implementation of the immune-inflammatory and oxidative stress markers measurements), doing the part analysis of the obtained data, interpretation of the obtained results, reviewing the literature, writing the manuscript and formulating answers for the reviewers.
*I estimate my contribution to be **30%.***

8. Depciuch J, **Sowa-Kućma M** (*first co-author; corresponding author),* Misztak P, Szewczyk B, Nowak G, Pankiewicz P, Parlińska-Wojtan M. Olfactory bulbectomy-induced changes in phospholipids and protein profiles in the hippocampus and prefrontal cortex of rats. A preliminary study using a FTIR spectroscopy.
Pharmacol Rep. 2016; 68:521-8. doi: 10.1016/j.pharep.2015.12.005
IF: 2.587 KBN/MNiSW: 25 Wydawnictwo: Elsevier
My contribution to this work concerned taking part in the plannig (selection of the research model) and the implementation of the study (experimenting on animals, preparation of samples for analysis), interpretation of the obtained results, reviewing the litaerature, preparation of the manuscript and formulation of responses for reviewers.
*I estimate my contribution to be **35%.***

C. Discussion of the scientific goal of the above work/works, the obtained results and their possible application

1. Scientific goal

Scientific research, described in the works listed in point IVB were aimed at determining the relationship between impaired functioning of ionotropic glutamate receptors (NMDAR) and zinc homeostasis as well as immune-inflammatory responses and oxidative stress in the etiology of affective disorders.

The series of publications presents the results of post-mortem (item 1, point IVB); clinical (items: 3-6, point IVB) and preclinical (items: 7 and 8, point IVB) studies and their complement is one review paper (item 2, point IVB).

Post-mortem study was partially supported by the grant no. KBN 6P05B14220. The majority of the remaining clinical (case-control) and preclinical studies were supported by grant no. POIG.01.01.02-12-004/09 (project Depression- Mechanisms-Therapy; DeMeTer; task 3.2; task leader: prof. dr hab. Gabriel Nowak) financed by the European Regional Development Fund. This project was implemented in cooperation with the Department of Psychiatry, Jagiellonian University Medical College and headed by prof. dr hab. Krzysztof Wędzony from Institute of Pharmacology, Polish Academy of Sciences in Krakow. Within the DeMeTer project, the author of the habilitation cycle played a key role in the: planning and implementation of the studies (development, methodology optimization and determination of oxidative stress and immune-inflammatory markers), statistical analysis, interpretation of the obtained results, reviewing the literature, preparation of the manuscript and formulation of responses for reviewers.

Case-control study included patients diagnosed with major depression (MDD, n = 114) and bipolar disorder (BD, n = 133) with varying severity of symptoms, both sexes and healthy volunteers (control group, HC, n = 50).

In preclinical studies, animal models such as: zinc deficiency and bilateral olfactory bulbectomy were used.

Before publication, the concept and results of the studies described in the series of publications were presented at national and international scientific conferences:

1. **8-13 Feb 2008** International Society for Zinc Biology 1st Annual Meeting, Banff, Canada ("Zinc an antidepressant or adjunct agent: preclinical and clinical data", Nowak G, **Sowa M**, Szewczyk B, Poleszak E, Siwek M, Dudek D, Papp M, Zięba A, Pilc A)
2. **20-22 May 2010** Sixty-Fifth Annual Scientific Convention and Meeting of the Society of Biological Psychiatry, New Orleans, Louisiana, USA ("Magnesium and glutamate interaction in depression and antidepressant therapy" Nowak G, **Sowa-Kucma M**, Poleszak E, Pilc A)
3. **16-18.09.2010** 17th International Congress of the Polish Pharmacological Society, Krynica Zdrój ("Zinc and magnesium interaction with glutamate system in depression", Nowak G., **Sowa-Kućma M**., Szewczyk B., Poleszak E., Pilc A)
4. **13-17 Oct 2012** Society of neuroscience 2012, New Orlean, USA ("Serum protein biomarkers in depression: clinical trials" Szewczyk B, **Sowa-Kucma M**, Siwek M, Dudek D., Styczen K., Witkowski L., Pilc A., Nowak G.)
5. **30 May 2014** 9th Young Physicists Conference, Rzeszow, Poland („The use of Raman spectroscopy and infrared spectroscopy to identify differences in serum blood composition of persons suffering from affective disorders" Depciuch J, **Sowa-Kućma M**, Parlińska-Wojtan M)
6. **15-19 Sep 2014** E-MRS Fall Meeting, Warsaw, Poland („The use of Raman spectroscopy and infrared spectroscopy to identify differences in serum blood composition of persons suffering from affective disorders" Depciuch J, **Sowa-Kućma M**, Parlińska-Wojtan M)
7. **22-23 Nov 2014** National Conference of Students and Doctoral Students, Lublin, Poland ("Changes in the level of TBARS, zinc and copper in affective disorders – clinical trials" Pańczyszyn-Trzewik P, Misztak P, Nowak G, **Sowa-Kućma M**)
8. **27–29 Jun 2014** EBPS Workshop Immune Influences on Brain & Behaviour: from Psychoneuroimmunology to Immuno- psychiatry, Brighton, United Kingdom ("Associations of depressive symptoms with serum concentrations of cytokines and cytokine receptors: clinical trials" **Sowa-Kućma M**, Misztak P, Styczeń K, Szewczyk B, Siwek M, Dudek D, Nowak G)
9. **23 Jan 2015** Szczecin Medical Physics Days, Szczecin, Poland ("The use of Raman spectroscopy and infrared spectroscopy to study the impact of zinc on the phospholipid-protein balance in the blood serum of rats" Depciuch J, **Sowa-Kućma M**, Parlińska-Wojtan M)
10. **07–11 Jul 2015** IBRO – 9th WORLD CONGRESS International Brain Research Organization, Rio de Janeiro, Brazil ("Affective disorders: role of immune-inflammatory cytokines, oxidative stress and micronutrients" Misztak P, **Sowa-Kućma M**, Styczeń K, Siwek M, Dudek D, Szewczyk B, Rzeczyński W, Nowak G) http://ibro2015.org
11. **16-18 Jun 2016** 45th Congress of Polish Psychiatrists, "Human and Family, and Mental Health", Katowice, Poland ("TBARS in unipolar and bipolar disorder" Siwek M, Dudek D, Styczeń K, **Sowa-Kućma M**, Misztak P, Szewczyk B, Nowak G, Rybakowski J), Abstract book, p. 99.
12. **16-18 Jun 2016** 45th Congress of Polish Psychiatrists, "Human and Family, and Mental Health", Katowice, Poland ("Zinc in unipolar and bipolar disorder" Styczeń K, Siwek M, Dudek D, **Sowa-Kućma M**, Misztak P, Szewczyk B, Nowak G, Rybakowski J), Abstract book, p. 104
13. **22-24 Apr 2016** Neuronus 2016. IBRO & IRUN Neuroscience Forum, Krakow, Poland ("Alterations in BDNF level in the frontal cortex of suicide victims are associated with NMDA and AMPA receptors changes" Pańczyszyn-Trzewik P, Misztak P, Nowak G, **Sowa-Kućma M; B**ook of Abstracts, www.neuronusforum. pl, 2016, p. 63)
14. **18-20 Mar 2013** 13th International Review of Bipolar Disorders, Sevilla, Spain ("Markers of oxidation stress in the context of bipolar disorder in comparison with unipolar disorder. Preliminary data" Siwek M, Dudek D, Styczeń K, Nowak G, Szewczyk B, **Sowa-Kućma M**)

The main objectives of the publications presented in point IVB were:

- Assessment of the concentration of zinc, its affinity for the NMDA receptor (NMDAR) and their correlation with changes in the NMDAR subunit composition (GluN2A, GluN2B) and the postsynaptic density protein (PSD-95) in the hippocampus of suicide victims.
- Determination of TBARS levels (oxidative stress/lipid peroxidation marker), immune-inflammatory markers as well as zinc in the the blood serum of MDD and BD patients, and healthy volunteers and their correlation with stages of illness.
- Determination of the effect of zinc-deficient diet on the level of oxidative stress (TBARS and PCC) and immune-inflammatory (Interleukin-1α and β) markers in the blood serum and brain structures (frontal cortex and hippocampus) of rats.
- Determination of changes in the lipid-protein profiles in the brain sections of bulbectomized rats (olfactory bulbectomy – an animal model of agitated depression; the main cause of suicides) using infra-red (FTIR) spectroscopy.

2. Introduction

2.1. Depression - symptoms and occurrence

Depression is one of the most common mental disorder, especially in highly developed countries. Each year, the number of depressed patients increases dramatically. Depression will have become the leading causes of social and economic disability in the modern world. The annual morbidity due to depressive disorders in the adults is between 6-12%, and among people over 65, it can be as high as 30% [Docherty et al., 2017]. Depression occurs already in infants, however the percentage of patients increases with age. It is particularly evident comparing the number of diagnoses in adolescences and people between 40 and 65 years [Ghandour et al., 2018]. The World Health Organization (WHO) have reported that nowadays more than 350 million people (globally, in all ages) suffer from depression as well depressive disorders account for nearly 4.3% of the global burden of diseases [Docherty et al., 2017]. The risk of first MDD (major depressive disorders) lifetime symptoms is estimated at 8-12%. Besides age, gender also seems to be a predisposing factor; indeed the prevalescence of depressive disorders is almost two-fold higher in female depressed subjects than in male subjects [Kuehner, 2017]. Depression can lead to suicide, and 15% of those who are clinically depressed die by suicide. Most of depressed patients have attempted suicide (20-60%) or have

suicidal ideations (40-80%). Suicide is the second leading causes of death among those aged 15-29, and the third in people on aged 15-44. Furthermore, understanding the connection between depression and suicidal behavior is significant issue [Bachmann, 2018].

Depression is still an important societal problem. In general, people want to keep in secret their psychiatric dysfunction. This contributes to diagnostic and pharmacological difficulties of the disease. Despite extensive research, the neuropathology of depression is poorly understood. Studies of antidepressant mechanisms and the development of more effective therapeutic agent have also progressed slowly. Indeed, more than 1/3 of patients are resistant (do not respond) to antidepressant treatment [Johnston et al., 2019; Czarny et al., 2018]. Importantly, most of antidepressants require long-term use, which is necessary to get therapeutic effect. Therefore, patients need to take their antidepressants for 2-3 weeks to relieve depressive symptoms [Boku et al., 2018]. This clinical and pharmacological problems results mainly from insufficient knowledge on the neurobiological substrates of depression.

2.2. The neurobiology of depressive disorders

Depression is a heterogeneous disease, the development of which may be supported by various factors: biological, social, genetic, psychological or somatic. Of these, biological factors seem to deserve special attention. Over the last several decades, many theories of the pathogenesis of depression have been described. However, the complexity and heterogenity of depression makes imposible its association with a single, universal pathophysiological mechanism [Liu et al., 2017].

Monoaminergic hypothesis and role of adaptive changes in the brain

The monoamine hypothesis of depression has assumed that depression is caused by decreased level of one or more of the monoamines, including: serotonin (5-HT), noradrenaline (NA) and dopamine (DA). This widely accepted theory has been postulated that depressive symptoms may be associated with metabolism disturbances of biogenic amines in brain structures responsible for cognitive and emotional processes. Based on this evidence, noradrenergic and serotonergic theories of depression have been described [Liu et al., 2017].

The importance of monoamines, especially in the short-term action of antidepressants (ADs), has long remained at the center of clinical interest and resulted in the development of a large number of ADs, which are still widely used. Their action was mainly based on the reuptake inhibition (selective serotonin reuptake inhibitors, SSRIs and selective serotonin and

noradrenaline reuptake inhibitors, SNRIs) or enzymatic activities responsible for the degradation of catechol and indole amines, resulting in an increase in NA, 5-HT or DA levels in the synaptic cleft, and enhances the conductivity in these neurotransmission systems [Liu et al., 2017]. Studies to prove the monoaminergic hypothesis of depression and the mechanism of ADs action in the presynaptic area have not clearly demonstrated that in patients with depressive disorders there is a real deficiency of monoamines in the brain. Acute treatment with ADs induce in animals several (e.g. behavioral, neurochemical) effects, but this alterations are not considered to be a causative determinant of antidepressant action. The clinical effects of ADs, regardless of their type, have been observed in patients after few days of use and did not evoke a characteristic common effect among neurotransmission systems [Doboszewska et al., 2017]. Furthermore, there is evidence that amphetamine and cocaine, the substances which increase the monoamine levels in the brain, have not shown antidepressant effects. On the other hand, decreased concentration of 5-HT due to reduced supply of its precursor (tryptophan), did not lead to development of depressive symptoms [Hillhouse and Porter, 2015]. All these observations have led to a change in the direction of research into the mechanisms of depression and increased interest in the influence of long-term ADs administration on the postsynaptic area in neuron. As a consequence, the original monoaminergic hypothesis has been modiefied and its new version underlined the importance of adaptative changes to a sudden increase in monoamine concentration in the synaptic cleft leading to a decrease or increase in the sensitivity of neurotransmitter system [Liu et al., 2017]. Unfortunately, numerous studies do not allow to determine satisfactorily whether any of the described adaptative changes have a direct impact on the occurrence of the therapeutic effect of ADs. So far, no single characteristic alteration has been identified that could be a common link in the way of antidepressant activity. On the contrary, the study on the adaptive changes gave rise to a number of concerns, since many antidepressants with high therapeutic efficacy, acted in a manner contrary to the accepted hypothesis. This data suggests, that studies of depressive disorders cannot be limited only to sensitivity and density changes in a number of monoamine receptors.

Activation of the hypothalamic-pituitary-adrenal axis

Subsequent conceptions regarding the biological basis of depression paid more attention to the phenomenon of long-term stress, the consequence of which is the dysfunction of the hypothalamus-pituitary-adrenal axis (HPA). Hyperactivity of the HPA axis leads to increased synthesis and release of glucocorticoids, especially cortisol, which under normal conditions

are suppressed by glucocorticoid receptors (GRs). It is known that these receptors are found mainly in the hippocampus and are involved in the mechanism of negative feedback (long loop from the adrenal glands to the hypothalamus and a short loop from the adrenal glands to the pituitary gland) [Boku et al., 2018]. Preclinical studies have demonstrated a reduced number of GRs in brain structures involved in negative feedback in stressed animals [Calfa et al., 2003], and increasing their density in the hippocampus after some ADs [Przegaliński and Budziszewska 1993]. It is estimated, that HPA axis dysfunction affects 50-70% of people diagnosed with depression. In patients, increased concentration of glucocorticoids in the plasma, urine and cerebrospinal fluid, changes in the circadian secretion of glucocorticoids, increase in the volume of pituitary or adrenal glands were observed [Hansson, 2015]. Of the brain structures, the hippocampus seems to be the most susceptible to stress-induced changes and HPA axis hyperactivation. Among others, a decrease in the brain-derived neurotrophic factor (BDNF) expression, weakness of long-term potentiation (LTP) and inhibition of neurogenesis in the hippocampus (especially in the dentate gyrus) were described. An increasing number of studies also shown an important correlation between the decreased hippocampal volume and co-occurrence of depressive episodes [Gałecki and Talarowska, 2018].

Glutamatergic hyperactivation and zinc dyshomeostasis

Another adverse effect of long-term exposure to stress factors is the increased release of glutamic acid [Takeda et al., 2016]. Glutamate is the primary excitatory neurotransmitter in the brain, which is found in substantially higher concentrations than monoamines and in more than 80% of neurons. The proper functioning of the glutamatergic system together with the antagonistic, inhibitory GABAergic system, determines the maintenance of homeostasis. The lack of balance between these neurotransmission systems is the basis of the theory of depression, according to which hyperactivity of the glutamatergic system can lead to development of the disease [Młyniec, 2015].

Serious evidence for the involvement of glutamatergic system in depression comes from numerous studies showing consistent abnormalities such as: an increase glutamate concentration in body fluids and tissues of depressed patients [Altamura et al., 1993; Mitani et al., 2006; Levine et al., 2000; Hashimoto et al., 2007]; changes in the activity and expression of the excitatory amino acid transporter (EAAT) or vesicular glutamate transporter (VGluT) [Bernard et al., 2011; Zink et al., 2010], as well as reduced level of glutamate/glutamine complex (Glx) in depressive patients [Yuksel and Ongur, 2010; Horn et al., 2010].

Implications of glutamate in pathophysiology of depression have proven in magnetic resonance spectroscopy (MRS) studies [Grimm et al., 2012; Ghasemi et al., 2014]. Moreover, scientific data indicates that antidepressants may regulated the glutamate release or function of glutamate receptors [Maes et al., 1998].

Glutamate exerts its effects through the stimulation of several glutamate receptor subtypes. The most important from the point of view of the glutamatergic theory of depression seem to be a N-methyl-D-aspartate (NMDAR) and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propanoic acid (AMPAR) receptors, which belongs to the family of ionotropic receptors, and together with kainate receptors play an important role in excitatory neurotransmission by mediating fast postsynaptic potentials [Młyniec, 2015].

NMDA receptors are found on the majority of glutamatergic synapses in the central nervous system, and the brain regions with their highest density are: the hippocampus, cerebral cortex and amygdala. NMDAR are located on both neural and glial cells, pre- or postsynaptically. In addition, NMDARs are also present in the extrasynaptic space and are known to be able to move within the cell membrane. The physiological role of the receptors is very diverse and their properties strongly depend on the subunit composition and interaction with other cell membrane proteins, including the postsynaptic density protein (PSD) [Hillhouse and Porter, 2015]. It has been proven that NMDARs over-activation may lead to neurodegeneration in central nervous system (CNS). Under physiological conditions, there are a number of mechanisms that protect the cell through the effects of NMDA hyperactivation. It is assumed that congenital or acquired, permanent or periodic failure of these mechanisms is associated with the activation of pathophysiological processes resulting in specific neurological or psychopathological symptomatology, including depression [Murrough et al., 2017].

Studies from the second half of the 90s showed that tricyclic antidepressants, serotonin reuptake inhibitors and monoamine oxidase inhibitors diminish the function of NMDA receptors; a similar effect was also observed after electroconvulsive therapy [Skolnick et al., 1996; Nowak et al., 1998]. This mechanism of action is explained by the decrease in the affinity of glycine to its binding site in the NMDA receptor complex and the weakening of glycine's ability to modulate glutamatergic sites [Martinez-Turillas et al., 2007].

Significant arguments supporting the glutaminergic concept of depression were also provided by studies indicating the therapeutic effect of substances that affect this system. The most interesting data concern the potential antidepressant effects of ketamine (a phencyclidine derivative, an NMDA antagonist). Ketamine (in low subanesthetic doses) induces a rapid

antidepressant response, even in treatment-resistant depression [Duman, 2018; Andrade, 2017]. Besides, antidepressant activity has also been demonstrated for other glutamatergic receptors ligands, such as: CP-101-606, memantine (NMDAR antagonist) or MK-801 [Młyniec, 2015]. In addition, numerous studies, including many carried out in our team, confirm the antidepressant activity of zinc [Nowak, 2015].

Zinc (Zn) is one of the most important trace elements in the human body and its dyshomeostasis can have a significant impact on pathological processes associated with the etiology of affective disorders. There is evidence that zinc deficiency is accompanied by psychopathological symptoms, largely coinciding with symptoms of depression [Wang et al., 2018]. In the central nervous system (CNS), zinc in a multidimensional manner affects the glutamatergic transmission and related neuroplasticity and excitotoxicity. The antidepressant action of zinc is related with their antagonistic activity toward NMDARs or metabotropic glutamate receptors (group I and II) and enhancement of AMPARs function [Młyniec, 2015]. Like some antidepressants and mood stabilizers, Zn inhibits glycogen-3 kinase synthase (GSK-3) [Siwek et al., 2013]. Preclinical studies have been shown that zinc administration induced antidepressant response by itself, while several case-control trials indicated antidepressant activity of zinc supplementation in depressed patients. Furthermore, numerous clinical data also confirm the role of zinc deficiency in the development of depressive disorders. A reduction of blood (serum, plasma) and brain zinc concentration in MDD patients and in animals models of depression was demonstrated. Some of studies show correlation between zinc deficiency and drug-resistance in patients [Siwek et al., 2013]. The presented changes are releated (most likely) with inflammation, acute phase response, and oxidative stress. These issues are widely discussed in the review paper by Siwek et al. (2013).

In summary, all of the above reports suggest the importance of NMDA receptors in the pathophysiology of depression and their potential therapeutic (or diagnostic) utility. Unfortunately, despite numerous studies in this area, the molecular mechanism by which glutamate and its receptors regulate depression-related behaviors is still not well understood. From the point of view of pathological processes (and the potential effectiveness of antidepressant therapy), changes in the NMDAR subunit composition (arrangement) seem to be particularly important. Similarly, the various post-translational modifications of subunit proteins, theh critically influence the activity of receptors and also determine their distribution /position in the synapse seem to be of great significance [Taniguchi et al., 2009; Knox et al., 2014].

Immune-inflammatory activation

Inflammation is most often associated with the body's response to injury or infection. Currently, a sufficient number of evidence confirms the fact that psychological stress can induce immune system response, manifested by increasing of certain substances - mediators of the immune response [Gałecki and Talarowska, 2018].

Already in the 1990ties it was show that depressive disordes are accompanied by sign of a Thelper (Th)-1 shift and inflammation. Both MDD and BD are accompanied by increased levels of soluble interleukin (IL)-2 receptor (sIL-2R) and interferon gamma (INF-γ) or peripheral blood mononuclear cells (PBMC) activation [Meas i wsp., 1990; 1991, 1992a, 1994; Seidel i wsp., 1995]. It was also observed that depression is associated with constant activation of the immune response manifested by an increase in IL-1β, tumor necrosis factor alpha (TNF-α), soluble receptor antagonist (sIL-1RA), IL-6 and acute phase proteins [Maes et al., 1991, 1992b, 1994, 1995a, b; Służewska et al., 1995; Frommberger et al., 1997; Mikova et al., 2001]. Later studies (including numerous meta-analyzes), strongly confirm the importance of immune cells and various cytokines in the pathomechanism of depression and reveal a high heterogeneity of the results [Howren i wsp., 2009; Dowlati i wsp., 2010; Liu i wsp., 2012; Kohler i wsp. 2017; Moughrabi i wsp., 2014; Rizavi i wsp., 2016].

The significance of inflammatory processes in depression is also indicated by the correlation between the use of antidepressant drugs and lowering the concentration of proinflammatory cytokines with simultaneous improvement of the clinical status of the patient [Maes et al., 1995a, Hannestad et al., 2011; Hiles et al., 2012]. On the other hand, it is suggested that elevated levels of IL-6, sIL-1RA, or TNF-α may be a marker of drug resistance [Maes et al., 1997; Lanquillon et al., 2000]. Confirmation of the significant share of pro-inflammatory cytokines to the development of depression is the resolution of its symptoms when the concentration of pro-inflammatory cytokines reaches the values recorded in healthy subjects [Katon et al., 2007].

Most studies on the activity of immune cells and the levels of inflammatory markers in depressive patients are conducted in peripheral tissues. Only few reports indicate changes in these parameters in the central nervous system (CNS). It seems, however, that the study of inflammatory processes in periheral tissues is a reflection of changes occurring in the CNS. It is known, that immune cells and products of their activation can affect the CNS tissues due to their ability to penetrate the blood-brain barrier. In addition, immunocompetent cells of CNS, such as glial and microglia cells, produce cytokines and have cytokine receptors [Kelley et al., 2007]. Summarizing, these data confirm the participation of both peripheral and central

inflammation in depression, but they do not explain the mechanisms that lead to its development.

Pro-inflammatory factors are considered as an important element of communication between the immune system (both peripheral and central), and the neurotransmitter system and the (neuro)endocrine system. Cytokines are recognized as an important modulator of neurotransmitters turnover, including key monoamines in mood regulation, such as 5-HT, NA or DA [Kitagami et al., 2003; Miller et al., 2008]. Widely discussed in the context of depressive disorders is the influence of pro-inflammatory cytokines on catabolism and the availability of plasma tryptophan - the primary prekursor of serotonin. It is known, that IL-1, IL-2 and INF-γ can enhance the activity of indoleamine 2,3-dioxygenase (IDO), an enzyme which catabolized tryptophan into kynurenine, which is further broken down into kynurenic acid and quinolinic acid (calledas TRYCATs - tryptophan catabolites) [Brown i wsp., 1989; Hu i wsp., 1995; O'Connor i wsp., 2009]. This is confirmed by the relationship between IDO activity, inflammatory markers concentration and the severity of depression [Maes et al., 2002]. One of the consequences of IDO activation is the reduction of the serotonin synthesis/turnover, on the other hand, the neurotoxic impact of tryptophan metabolites on cells is observed [Oxenkrug, 2010]. While kynurenic acid is the NMDAR endogenous antagonist, quinolinic acid is a strond agonist of this receptor. Furthermore, TRYCATs can also activate inducible nitric oxide synthase (iNOS) and thereby increase the synthesis of nitric oxide (NO). NO, apart from its important physiological function, is also a reactive oxygen species (ROS). The disturbance of the balance between pro-oxidative and antioxidant factors in the cell (referred to as oxidative stress), leads to disturbances in the structure and function of cell, and consequently, its death. CNS is particularly vulnerable to oxidative damage, due to: high metabolic activity; lower activity of antioxidant enzymes; a high content of unsaturated fatty acids - substrates for oxidation; redox potential of a number of neurotransmitters or a high concentration of metal ions (e.g., iron, copper) involved in redox reactions. Furthermore, oxidative stress can damage the central nervous system by induction of exitotoxicity mechanisms (uncontrolled influx of calcium into the cell) mediated by glutamate and hyperstimulation of NMDA receptors [Gutteridge and Halliwel, 2018; Ng et al., 2008]. Calcium (Ca) homeostasis in the cell is maintained by the mitochondria (responsible for energy metabolism), which are cellular Ca reservoirs. An increase in Ca^{2+} ion concentration may lead to irreversible damage to mitochondria as a result of the intensification of free radical processes and imbalance between antio- and pro-oxidants processes in the cell [Halliwell, 2006, 2011]. Another key element of this whole "puzzle" is

zinc, which prevents over-activation of NMDAR, and on the other hand, has antioxidant and anti-inflammatory activity, mainly as a co-factor of numerous enzymes [Prasad, 2014].

Inflammatory processes seem to be equally important for the functioning of the endocrine system, in particular the HPA axis [Hayley, 2011]. Under normal conditions, HPA axis inhibit the synthesis of pro-inflammatory agents by secretion of cortisol, while inflammation is accompanied by a disturbance in the functioning of this feedback loop, which is crucial for the correct response to stress factors. This is because immune cells become less sensitive to the anti-inflammatory effects of glucocorticoids due to the decreased expression of their receptors. This phenomenon is often observed in patients with depressive disorders [Gałecki and Talarowska, 2018].

The presented effects of inflammatory markers allow to indicate these molecules as primary factors in depression. Due to the fact that the peripheral and central inflammatory systems function in parallel, the analysis of inflammatory markers concentrations in the peripheral blood should be a reliable determinant of the activation of immune cells in the brain.

2.3. Justification of the habilitation thesis

The aforementioned classification shows, in a very concise way, how over the past 50 years, knowledge about the cellular mechanisms involved in the development of depressive disorders has changed. However, it does not include a holistic approach to seeking the causes of depression in individuals, which should be comprehensive and take into account their complexity and pathogenesis of depression in human. The most important theories of depression only selectively focus on several cellular pathways, while omitting others. In addition, available literature data often provide divergent information, which is usually a consequence of the high heterogeneity of the studied groups and the analytical techniques. All this makes it difficult to draw constructive conclusions that could be translated into clinical (both diagnostic and therapeutic) usefullness.

Among the presented hypothetical mechanisms of depression, it seems that inflammation accompanied by the glutamatergic system hyperstimulation, exacerbation of exotoxicity and oxidative stress, with simultaneous weakening of anti-inflammatory and anti-oxidant systems (e.g., zinc deficiency), plays a leading role in the pathophysiology of depression. Despite numerous clinical and preclinical studies, there is no hard evidence to support this hypothesis so far. Therefore, the aim of the presented series of works submitted for evaluation was to determine the relationship between impaired functioning of NMDARs

and zinc homeostasis, as well as oxidative and inflammatory processes in the etiology of affective disorders.

3. Results

The series of scientific publications designated as achievements for habilitation proceedings consists of 8 works, published in the years 2013–2018, entitled "Zinc, NMDA receptors, oxidative stress, inflammation and their relationships in the pathophysiology of depression" in journals listed in the Journal Citation Reports.

The research being the subject of the presented cycle of works included 4 thematic issues.

Issue 1. Analysis of the zinc concentration, its affinity for the NMDA receptor and their correlation with changes in the subunit composition (GluN2A, GluN2B) of the NMDAR and the postsynaptic density protein (PSD-95) in the hippocampus of suicide victims.

The main aim of the study presented in the paper by **Sowa-Kućma et al. (2008, item 1, point IVB)** was to verify the hypothesis that there is a decreased concentration of zinc and reduced its potency to inhibit NMDA receptor activity (measured as the ability of zinc to inhibition of [^{3}H] MK-801 binding to NMDAR) in the hippocampus of suicide victims. The research was carried out on post-mortem tissues obtained at the time of autopsy from suicide victims (n = 17) and age-matched controls (sudden death, n = 6).

The obtained results showed a reduced affinity of zinc to the NMDA receptor, observed as increase (by 29%) in the IC_{50} value of zinc inhibition of [^{3}H] MK-801 binding to NMDAR between the control and suicide tissue. This findings were consistent with previous study published by our team [Nowak et al., 2003], which revealed that the same changes in the interaction between zinc and NMDA mihgt be involved in the psychopathology underlying suicidality. On the other hand, measurement of zinc concentration in the same tissues did not show any change. In this respect, it seemed interesting to investigate what may be the reason of the reduced affinity of the zinc to NMDAR. It is known that the NMDAR functions as a heterotetramer of two glycine-bnding GluN1 subunits and two glutamate binding GluN2 subunits, and its arrangement can vary depending on the current state of the cell and have a very large impact on the biophysical and pharmacological properties of the receptor. Given the fact that MK-801

binds with the same affinity to the GluN2A and GluN2B subunit, while zinc (depending on its concentration) with different affinities to the both subunits, in the next step of this study we determine the level of these proteins using the Western Blot method. At the same time, we determined the level of PSD-95 protein, which are responsible for anchoring the receptor in the cell membrane.

The obtained results indicato that the amount of GluN2A is significantly elevated (68%) in the hippocampus of suicide subjects when compared to sudden death controls. On the other hand, the GluN2B and PSD-95 protein levels were decreased (by 46% and 36%, respectively). Thus, the reduced potency of zinc to inhibit the NMDAR was accompanied by an increase in GluN2A expression and a decrease in GluN2B. This pattern of changes in the GluNA vs. GluN2B subunit level may also explain the lack of differences in specific binding of [^{3}H] MK-801 to NMDAR between the suicide and control groups. Likewise, binding studies using a variety of radioligands showed no alterations only in glutamate sites with no changes in the ion channel or glycine sites of NMDA complex in brain samples from suicides [Palmer et al., 1994; Dean et al., 2001; Meador-Woodruff et al., 2001]. The results obtained by other authors confirm the significance of changes in the NMDAR subunits composition in the pathophysiology of depression. For example, Nudmamud-Thano and Reynolds [2004] have demonstrated reduced expression of GluN1 subunit in the temporal cortex of both MDD and BD patients. There was also a decreased of GluN2A, GluN2B and PSD-95 protein level in the frontal cortex [Feyssa et al., 2009], increased of GluN2A and PSD-95 in the amygdala [Karolewicz et al., 2009] as well as GluN2C in the locus coeruleus [Karolewicz et al., 2005] of depressed patients.

The altered function/composition of NMDAR complex in the pathophysiology of depressive disorders also confirm studies on experimental models of depression. Similarly to human brain tissues, these changes also seem to be multidirectional [Pochwat et al., 2014].

Issue 2. Determination of TBARS levels (oxidative stress/lipid peroxidation marker), immune-inflammatory markers as well as zinc in the the blood serum of MDD and BD patients, and healthy volunteers and their correlation with stages of illness.

All studies presented in the next 4 publications (items 3-6, point IVB) were performed on human blood serum collected from patients diagnosed with major depressive disorder

(MDD) or bipolar disorder (BD) and healthy volunteers (HC). The study enrolled a total of 347 subjects, including 114 MDD patients, 133 BD patients and 50 healthy volunteers. Among BD group, 69 persons were diagnosed with type I (BDI), while 64 represented type II (BDII). All patients fulfilling the DSM-IV-TR criteria for MDD or BD (both in the active phase of depression, and achieve remission) were recruited to the case-control study. The most important exclusion criteria were: diagnosis of a severe psychiatric disorders other than MDD/BD e.g. schizophrenia), substancje use disorders (excluding addiction to nicotine and cafeine), comorbidity of serious physical illness, pregnancy, obesity, occurrence of inflammatory diseases, use of non-steroidal anti-inflammatory drugs and others. For the measurement of the severity of depressive symptoms the Hamilton Rating Scale for Depression (HDRS) and Montgomery-Asberg Depression Rating Scale (MADRS) were used. The severity of mania symptoms in BD was determined using the Young Mania Rating Scale (YMRS).

In the collected blood serum, an measurement of Thiobarbituric Acid-Reactive Substances (TBARS; byproduct of lipid peroxidation) were performed. The detailed analysis of the research on the relationships between depressive disorders and oxidative stress shows that the increased level of malondialdehyde (MDA) or TBARS (the product of MDA reaction with thiobarbituric acid) is the most frequently observed phenomenon in depression reflecting oxidative stress **[**for review see; **Siwek et al. 2013 (item 2, point IVB)].**

Among the inflammatory markers widely discussed in the literature, we have decided to measure concentrations of several cytokine receptors: sIL-1RA, sIL-2R, sIL-6R, sTNF-R1 (60) and sTNF-R2 (80), rather than their cytokine counterparts (except IL- 1α) because measurements of the receptor levels are more reliable, while showing higher levels than cytokines, which are often not detectable in peripheral blond (e.g. IL-2). We are also interested in IL- 1α levels as many papers in depression focused on IL- 1β while neglecting IL- 1α. In addition, certain factors (e.g. sIL-1RA) also indicates the activation of compensatory anti-inflammatory mechanisms, which allows a broader look of the whole issue.

Measurement of zinc concentration in the same study participants was the complement of the TBARS and immune-inflammatory markers analysis.

The first major finding presented in the paper by **Sowa-Kućma et al. (2018, item 3, point IVB)** is that major depressive disorder is accompanied by increased sIL-1RA, sTNF-R1 and TBARS levels as compared to normal controls. Melancholic depression is associated with increased sIL-6R but lowered IL-1α levels. A current episode of depression is accompanied

by significantly increased sIL-6R compared to the remitted state. Treatment-resistant (TRD) depression is characterized by increased sIL-6R and TBARS but lowered sTNF-R2 levels in relation to non-TRD patients. These immune markers are not significantly correlated with HDRS, MADRS, number of episodes, or age of onset.

The zinc concentration in the serum samples in depressive episode were significantly lower from those observed in the health volunteers group (**Styczeń et al., 2017; item 4, point IVB).** However, there were no statistically significant differences in zinc levels between ptients achieve remission and control group or between depressed stage and remission. Among the group of depressed patients there was no statistically significant difference in zinc levels between patients with and without the following clinical features: atypical or psychotic symptoms of depression, melancholic syndrome, or drug-resistance. On the other hand, among group of patients in the remission a significant differences in zinc concentration between patients with and without presence of drug-resistance in the previous episode of depression were observed. Morover, our findings demonstrated no significant correlations between zinc levels and age of patients and some clinical features (for all: patients in depressive chase, remission and Total group population): duration of the disorder, average number of hospitalizations or depressive episodes in the last year, number of hospitalizations throughout life, severity of depression measured by HDRS, or MADRS or duration of current episode severe depression, or remission. The only significant correlation was obtained between zinc concentration in patients achieve remission and average number of depressive episodes in the last year.

Analysis of zinc concentrations in patients diagnosed with bipolar disorder **(Siwek et al., 2016, item 5, point IVB)** also indicates its significant reduction in the depressive phase in relation to both healthy volunteers and patients in the mania/hypomania phase and achieve remission. Differentiation of patients into two groups: BD I and BDII revealed significantly reduced level of zinc in BDI subjects in depressive phase as compared to healthy people and patients in mania/hipomania and no differences between the depressive and remission phase. Furthermore, in the BDII group, no differences were observed between the various stages of disease development.

Due to the fact that the assessment of the above-mentioned serum oxidative and immune-inflammatory markers in all study participants was performed simultaneously, using the same analytical methods, it was possible and interesting to assess the potential usefulness of the investigated parameters to differentiation MDD and BD. Thus, the aim of the next publiaction (**Sowa-Kućma et al., 2018; poz. 6, pkt. IVB**) was detailed (taking into account different

clinical features) comparison of concentrations: TBARS, sIL-2R, sIL-6R, Il-1α, sIL-1RA and sTNF-R1/2 between MDD and BD patients using advanced statistical methods. For example, we transformed the immune and TBARS data into z scores and consequently computed z-unit weighted composite scores to make indices of 5 cytokine receptors (zCytR), cell-mediated immunity zCMI (zCytR + IL-1α) and immune-oxidative stress (zCMI+TBARS).

Obtained results revealed no significant differences in biomarkers between MDD and BD patients. Interestingly, the occurrence of atypical features in both groups was positively correlated with the sIL-6R and TBARS levels, whereas melancholia was associated with higher TARS and sTNF-R1 concentratios. SEverity of illness, as measured withe the HDRS, was correlated with increased sIL-6R, sTNF-R2, TBARS, zCMI and zCMI+TBARS. The number of episodes the year prior to blond sampling was positively associated with sTNF-R2, TBARS, zCMI and zCMI+TBARS, while number of hospitalizations was positively associated with sIL-1RA. Prior suicidal attempts were accompanied by elevated Wcześniejsze sIL-1RA, IL-1α, zCMI, TBARS i zCMI+TBARS, while TBARS was associated with current suicidal ideation.

Issue 3. Determination of the effect of zinc-deficient diet on the level of oxidative stress (TBARS and PCC) and immune-inflammatory (IL-1α and IL-1β) markers in the blood serum and brain structures (frontal cortex and hippocampus) of rats.

The main aim of the study presented by Doboszewska et al. **(2016; poz. 7; pkt IVB)** was to verify the hypothesis that zinc deficiency induces inflammation and oxidative stress in the CNS, which results in the development of symptoms of depression (in animals depressive-like symptoms) in humans.

The study was performed on rat tissues (frontal cortex, hippocampus, blood serum) collected from animals subjected to a zinc-deficient (3 mg Zn/kg, experimental group, ZnD) diet or a zinc-adequate (50 mg Zn/kg, control group, ZnA) diet for 4 or 6 weeks. The obtained results indicate the intensification of free radical and inflammatory processes in experimental animals. A 4-week of zinc-deficient diet caused an increase in TBARS level and carbonyl protein content (PCC; oxidative protein damage) both in the frontal cortex and hippocampus of rats. These changes were associated with increase in IL-1α and IL-1β concentration and decrease in zinc level in blood serum and brain structures. Interestingly, the concentration of TBARS in the blood serum of these group was reduced. In turn, the 6-week zinc-deficient diet induced an even greater increase in TBARS level in the frontal cortex and hippocampus of

rats, while the serum concentration remained unchanged compared to the ZnA group. ZnD animals also had a statistically significant lowered serum zinc level compared to the control group, while there was no alterations in both brain structures.

The above observations confirm the fact that the nervous system is more susceptible to oxidative/inflammatory damage, therefore the changes appear there earlier. Moreover, we can assume that there are some peripheral compensatory mechanisms that are weakened over time.

Likewise, no differences in the brain's zinc level after prolonged use of the zinc-deficient diet may suggest the activation of some adaptive mechanisms to long-lasting zinc deficiencies. This observation may also partly explain why in the paper by **Sowa-Kućma et al. (2008, item 1, point IVB)** there were no changes in the zinc concentration in suicide victims.

What is equally important, the observed changes were accompanied by behavioral changes (e.g. increased immobility time in the forced swim test, anhedonia, decreased social interactions), indicating the occurrence of depressive-like symptoms in rats. In addition, numerous biochemical changes in the rat brain were noted. The most important include the increase in GluN2A level and the reduction of BDNF level in the frontal cortex and hippocampus [Doboszewska et al., 2015].

In conclusion, all these dependencies clearly indicate that the primary zinc deficiency can lead to a number of peripheral and central changes in the body, which consequently lead to the development of depressive disorders.

Issue 4. Determination of changes in the lipid-protein profiles in the brain sections of bulbectomized rats (olfactory bulbectomy – an animal model of agitated depression; the main cause of suicides) using infra-red (FTIR) spectroscopy.

Obtained clinical and preclinical results strongly confirm that the inflammation accompanied by oxidative stress and disturbance of cellular proteins, may play a key role in the pathophysiology of depressive disorders, which may suggest the potential usefulness of their markers for diagnostic purposes (diagnosis of disease, differentiation of affective disorders, or monitoring disease progreession). The literature data and own experience show that the determination of selected protein markers often causes large analytical problems due to their relatively low concentrations (e.g. IL-2), which makes impossible to use commercial

sets for their determinations (low sensitivity of sets - detection impossible). In addition, it is difficult to identify one specific factor for the analysis, based on which it would be possible to draw unambiguous conclusions. Therefore, it would be necessary to analyze more markers using various analytical methods, which in turn would generate relatively high diagnostic costs. Taking this into account, the next goal of my study was to find an alternative method to identify alterations in biological tissues that would indicate the development of depressive disorders (or allow their differentiation) and correlate with the changes that we have shown in previous studies. In cooperation with the Institute of Nuclear Physics in Krakow, we have attempted to apply Fourier Transform Infrared Spectroscopy (FTIR) with Attenuated Total Reflectance (ATR) to identify changes in the brain strucutres of bulbectomized rats (olfactory bulbectomy – well-validated animals model of depression) (**Depciuch i wsp., 2016; poz. 8; pkt IVB**).

FTIR spectroscopy give complete information about the chemical composition of the studied samples. This cheap and quick tevchnique give repeatable measurement results of the analyzed biological material. Therefore, FTIR, together with other spectroscopic techniques (e.g., Raman and UV-vis spectroscopy) increasingly being used in biomedical sciences, for example, to identify changes associated with cancer in human. Our team, as first, used FTIR spectroscopy for the search markers of depressive disorders.

Surgical removal of olfactory bulbs induces in rodents the symptoms that mimic agitated depression (the main cause of suicide) in humans. Repeated administration of antidepressants (e.g. amitriptyline) reverses the OB-induced behavioral effects. In order to verify whether peripheral changes observed in previous studies correspond to changes in the brain of model animals, in the presented study we performed spectrometric analyzes in the rat brain sections. The obtained results indicate a signisicant reduction in the intensity of peaks corresponding to the functional groups belonging to phospholipids and proteins in the FTIR spectrum in bulbectomized (OB) rats compared to control animals (Sham) both in the frontal cortex and hippocampus.

Infrared spectroscopy is one of the most versatile research techniques that allows to observe the secondary structure of proteins and its changes induced by various external factors. Its unquestionable advantage is the ability to adjust the measurement conditions to a specific problem. Calculation of the second derivatives of the FTIR spectra allows a detailed comparison of changes in the components of the proteins and phospholipids structure as well. Our analyzes confirmed that olfactory bulbectomy affects not only the level, but also the structure of the tested compounds, which may possibly indicate their damage.

The 14-day treatment with amitriptyline (AMI) did not significantly affect the disturbed lipid balance in the OB group and at the same time caused an increase in protein levels. Wherein, a stronger effect of the drug was observed in the hippocampus. Similarly, the second derivative of the FTIR spectrum showed a positive effect of AMI administration on the structure of proteins (but not phospholipids), which would indicate its normalization after ADs injection. Our results suggest that in the course of depression there is a permanent (irreversible?) changes in both the level and structure of phospholipids, and reversible changes in proteins.

Phospholipids as the key building block of the cell membranes affect their structure and functions, also through their interaction with membrane proteins [Farkas et al., 2002; Skosnik and Yao, 2003]. Interestingly, changes in phospholipid levels positively correlated with alterations observed in the bands corresponding to protein functional groups. Our research suggests a significant disturbance of the amount (probably also function) of cellular phospholipid as well as proteins in the course of depression in humans, which may be, for example, a reflection of the disturbed function of neurotransmitter systems or the consequence of oxidative damage of cellular structures. These results provide new, interesting information on the pathomechanism of depression and indicate the potential usefulness of the spectroscopic techniques in the search for endogenous markers of depression.

The above-mentioned findings clearly indicate a disturbed phospholipid-protein balance in depression. The confirmation of this hypothesis are our two further studies using animal models of depression. In the paper Depciuch et al. (2017a), we showed that chronic mild stress not only alters (damages ?) the structure of phospholipids and proteins, but also lowers their level in blood serum of rats. Obtained information on the proteins and phospholipids structures using UV-vis spectroscopy combined with the second derivatives of the FTIR spectra, indicate the normalization of stress-induced changes in protein structure after administration of imipramine, with no effect on the structure of phospholipids. The same direction of changes was also observed in the study by Depciuch et al. (2017b) in serum of rats receiving zinc-deficient diet (ZnD; 3 mg Zn/kg) for 6 weeks relative to the control group (ZnA; 50 mg Zn/kg) and those injected repeatedly with amitriptyline (10 mg / kg/day). What is important, observations made in animals have also been confirmed in studies on the blood serum of patients diagnosed with depression (Depciuch et al., 2016). It is interesting to note that the changes observed in the brain are reflected in the blood, which means that they can be successfully analyzed outside the central nervous system.

In summary, our findings strongly suggest disturbed lipid-protein balance in the course of depression. Regardless of the type of factors that induce depression, permanent (irreversible) changes in the structure of phospholipids have been observed, which, unlike proteins, tend to be more susceptible to their negative effects. Taking into account the fact that lipids constitute as much as 60% of the dry mass of the brain, their impaired functioning will have a significant impact on the functioning of the nervous system and the whole organism.

Our research also shows new possibilities of applying spectroscopic techniques in the diagnosis and monitoring of human diseases. On the other hand, they point to new directions of searching for markers of affective disorders and are a part of a very fashionable trend in biospectroscopy.

4. Conclusions

Based on the presented results, the following can be concluded:

- Reduced ability of zinc to inhibit the NMDA receptor function in the hippocampus, which probably results from its rearrangement (changes in the subunit composition) is potentially involved in the pathophysiology of suicie-related disorders (e.g. depression).
- Elevated sIL-1RA, sTNF-R1, and TBARS levels may be a trait markers of depression, while increased sIL-6R concentration may be a state marker of melancholia and an acute phase of depression. Furthermore, elevated levels of sIL-6R and TBARS, and reduced sTNF-R2 concentration may be a potential markers of treatment-rresistant depression in MDD patients.
- There are no inflammatory and oxidative stress markers differences between MDD and BD, thus they can not be used to differentiate these affective disorders. However, the obtained results, clearly indicate that the severity of depression, number of episodes and suicidal attempts are associated with activated inflammatory and oxidative stress pathways.
- Among the analyzed peripheral blond markers, elevated TBARS level is the the single best predictor of MDD/BD, atypical depression, melancholia and current suicidal ideation. Therfore, novel antidepressant treatment should target oxidative stress pathways.

- Decreased serum zinc concentrations may be a trait marker of depression in MDD and BD patients (especially BDI) and a treatment-resistant depression marker in MDD.
- The experimentally induced zinc deficiency intensifies inflammatory and oxidative stress processes, especially in CNS, which partially correlates with changes observed in the peripheral blood. This confirms the key role of zinc as an antioxidant and anti-inflammatory agent. On the other hand, our results confirm the hypothesis that the CNS is more sensitive to inflammation and oxidative stress.
- In the course of depression, the disturbance of the (phospho) lipid-protein balance (manifested by a decrease in the level and structural changes of phospholipids and proteins) in the brain (and in the blood) is observed. Similar alterations were observed in ZnD animals, which may indicate that impaired zinc homeostasis may be crucial for the development of depressive disorders.
- Reversible changes in the level and structure of proteins observed after antidepressants may be a marker of the effectiveness of pharmacotherapy.
- Modern spectroscopic techniques (Raman, FTIR and UV-vis sepctroscopy) can be successfully used to identify the changes accompanying various human diseases, including depressive disorders. These techniques can be used to examine both the blood and other tissues. In future, spectroscopic techniques can serve as excellent new diagnostics methods of mental illnesses.

The series of scientific publications designated as achievements for habilitation confirm the relationship between the disturbed functioning of the glutamatergic system, zinc homeostasis as well as inflammatory and oxidative processes in the pathomechanism of depression. Our research has been a continuation of research conducted on this topic for many years. In contrast to previously published, for the first time, we clearly point to the multi-dimensional relationship between the studied changes/processes, which allows us to infer the potential universality of the investigated mechanisms. This was facilitated by comprehensive research, that we carried out simultaneously in the brain tissue and blood serum; in *post-mortem* tissues, blood collected from patients diagnosed with depressive disorders, as well as tissues of animals with depressive-like symptoms. The use of various analytical techniques, also not used in this type of research so far, has also enabled us to verify the correctness of the results obtained. At the same time, we are aware of some imperfections and the need for

further research. *Post mortem* studies should be carried out on a larger number of tissues obtained from patients with psychiatric diagnosis and a more accurate medical history, nevertheless, our study are among the few performed in the hippocampus. The presented case-control study should be extended in the future by cross-sectional study. What is worth emphasizing, we included in our study a relatively large number of patients of both sexes, with a diversified diagnosis, including detailed clinical parameters and features, which undoubtedly enriches the current knowledge about the pathophysiology of depression with new aspects. The presented cycle of publications provides further data that reinforce the hypothesis that link the pathophysiology of depressive disorders with allostatic load, as a combination of activation of inflammation, oxidative stress and excitotoxicity, remaining in mutual interaction. Our analyzes belong to a wide range of research, which nowadays allows first and foremost an insight into the biological mechanisms underlying the disease. In the future, they may enable the creation of clinically useful laboratory diagnostic systems and designate new directions for the search of novel antidepressant pharmacotherapies.

5. References

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6. Total impact factor and Hirsch index according to the Web of Science database (WoS)

My hitherto scientific achievements are presented in 56 publications (**49 original papers and 7 review papers**) which, according to the Web of Science Core Collection database, **have been cited 1120 times (without self-citations: 973 times**); all of them appeared in journals with an Impact Factor, included in the Journal Citation Reports database (**Total Impact Factor equals 159.295**). **7 original papers and 1 review paper forming my habilitation work (Total Impact Factor equals 24.593).** My **Hirsch index equals 19** (on 6 December 2018). The above statistics are higher than the average in my scientific discipline in Poland.

V. DESCRIPTION OF THE REMAINING SCIENTIFIC ACHIEVEMENTS

A. Scientific achievements before and after getting Ph. D. degree in medical sciences

Total number of publications from Journal Citation Reports database: 56

- number of publications **before obtaining a doctoral degree**: 10 (including 9 original papers and 1 review paper; Total Impact Factor equals **25.772**)
- number of publications **after obtaining a doctoral degree**: 46 (including 40 original papers and 6 review papers; Total Impact Factor equals **133.523**); without publications included in the habilitation cycle: 38 (including 33 original papers and 5 reviews papers; Total Impact Factor equals **108.93**)

My remaining scientific achievements, not being part of my habilitation work, include:

1. Determination of the cellular mechanisms involved in the antidepressant-like activity of zinc

Zinc is a key trace elements in the body that play an important role in most life processes. The highest concentration of this biometal is found in the central nervous system. It is well proven, that zinc plays crucial role in the regulation of neurotransmission. In particular, zinc acts as a modulator and a potent antagonist of NMDAR, thus modulates glutamatergic transmission. The first preclinical studies (e.g. forced swimming test, tail suspension test or experimental model of depression - olfactory bulbectomy) conducted by the prof. G. Nowak's team in the period 2000-2003 have shown antidepressant-like activity of zinc. Subsequent studies not only confirmed its antidepressant potential, but also showed that zinc suplementation of imipramine treatment significantly reduced depression scores and improved treatment outcomes in drug-resistant unipolar patients. Further study aimed to determine the molecular mechanisms associated with antidepressant-like activity of this biometal. For this purpose, a panel of tests (both behavioral and biochemical) was carried out using various research techniques (e.g. *in vitro* microdialysis, histochemical detection of synaptic zinc, Northern Blot, Western Blot, radioligand binding) and animal models of depression (including chronic mild stress and chronic unpredictable stress).

The most important conclusions from the research being the subject of the listed below paper are:

- antidepressant-like zinc activity is associated with an increase in the synaptic and extracellular zinc level in the frontal cortex and hippocampus
- antidepressant-like effect of zinc in the chronic mild and chronic unpredictable stress (animal models of depression) is associated with an increase in the brain-derived neurotrophic factor (BDNF) level
- chronic administration of zinc results in a decrease in the affinity of glycine to NMDA receptors in the frontal cortex and an increase in the density of 5-HT1A and 5-HT2A serotonin receptors in the frontal cortex and hippocampus
- both NMDA and AMPA receptors are involved in the antidepressant action of zinc; zinc activity was antagonized by the administration of N-methyl-D-aspartic acid, whereas NMDA receptor antagonists enhanced the effect of inactive doses of zinc. A similar effect was seen after administration of AMPA receptor potentiators.

Szewczyk B, ***Sowa M**, Czupryn A, Wieronska JM, Branski P, Sadlik K, Opoka W, Piekoszewski W, Smialowska M, Skangiel-Kramska J, Pilc A, Nowak G.:*Increase in synaptic hippocampal zinc concentration following chronic but not acute zinc treatment in rats.* Brain Res. 2006;1090:69-75. (IF 2.341; MNiSW 20)

*S**owa-Kućma M**, Legutko B, Szewczyk B, Novak K, Znojek P, Poleszak E, Papp M, Pilc A, Nowak G.**:** *Antidepressant-like activity of zinc: further behavioral and molecular evidence.* J Neural Transm. 2008;115:1621-1628. (IF 2.514; MNiSW 20)

Opoka W, ***Sowa-Kućma M,** Kowalska M, Baś B, Gołembiowska K, Nowak G.**:** *Intraperitoneal zinc administration increases extracellular zinc in the rat prefrontal cortex.* J Physiol Pharmacol. 2008; 59: 477-487. (IF 2.631; MNiSW 20)

Cichy A, *__Sowa-Kućma M__, Legutko B, Pomierny-Chamioło L, Siwek A, Piotrowska A, Szewczyk B, Poleszak E, Pilc A, Nowak G.: *Zinc-induced adaptive changes in NMDA/glutamatergic and serotonergic receptors.* Pharmacol Rep. 2009;61:1184-1191. (IF 2.086; MNiSW 20)

Szewczyk B, Poleszak E, ***Sowa-Kućma M**, Wróbel A, Słotwiński S, Listos J, Wlaź P, Cichy A, Siwek A, Dybała M, Gołembiowska K, Pilc A, Nowak G.: *The involvement of NMDA and AMPA receptors in the mechanism of antidepressant-like action of zinc in the forced swim test.* Amino Acids. 2010;39:205-217. (IF 4.106; MNiSW 27)

***Sowa-Kućma M**, Kowalska M, Szlósarczyk M, Gołembiowska K, Opoka W, Baś B, Pilc A, Nowak G.: *Chronic treatment with zinc and antidepressants induces enhancement of presynaptic/extracellular zinc concentration in the rat prefrontal cortex.* Amino Acids. 2011;40:249-258. (IF 3.248; MNiSW 30)

Cieślik K, ***Sowa-Kućma M (**corresponding author), Ossowska G, Legutko B, Wolak M, Opoka W, Nowak G.: *Chronic unpredictable stress-induced reduction in the hippocampal brain-derived neurotrophic factor (BDNF) gene expression is antagonized by zinc treatment.* Pharmacol Rep. 2011;63:537-543. (IF 2.445; MNiSW 25)

2. The role of zinc deficiency in the induction of depressive-like symptoms

Numerous clinical and preclinical data confirm the role of zinc in the pathophysiology and treatment of depression. It is suggested that zinc deficiency may lead to development of depressive symptoms in a course of major depressive disorder. Therefore, the aim of our research was to verify this hypothesis, as well as an attempt to develop a new animal model of depression based on a limited supply of zinc in the diet.

Our first studies (Opoka et al., 2010, Młyniec et al., 2012) showed a significant, time-dependent effect of low-zinc diet on the behavior of animals (changes in spontaneous locomotor activity and immobility time) which was correlated with alterations in serum zinc and corticosterone concentration.

Subsequent studies confirmed our preliminary observations. Doboszewska et al. (2015a) described a number of behavioral changes (referred to as depressive behavior) after 4 and 6 weeks of zinc deficiency (3 mg Zn/kg) diet in rats. Among others, increased immobility time in the forced swimming test, lower intake of 1% sucrose solution (anhedonia) and reduction of social behavior have been demonstrated. Observed changes were accompanied by increase in the NMDAR subunits (GluN2A and GluN2B) level and decrease in PSD-95, p-CREB and BDNF protein in the hippocampus. All these results indicate disturbed glutamatergic transmission and brain plasticity in the conditions of decreased zinc supply. Interestingly, the 14-day administration of antidepressant drug - fluoxetine (10 mg/kg) reversed the zinc deficiency-induced behavioral changes and partly also biochemical alterations (the level of GluN1, GluN2A and GluN2B subunits, but not BDNF) in the hippocampus of rats (Doboszewska et al. 2015b).

Opoka W, ***Sowa-Kućma M,** Stachowicz K, Ostachowicz B, Szlósarczyk M, Stypuła A, Młyniec K, Maślanka A, Baś B, Lankosz M, Nowak G.: *Early lifetime zinc supplementation protects zinc-deficient diet-induced alterations.* Pharmacol Rep. 2010;62:1211-1217. (IF 2.500; MNiSW 27)

Młyniec K, Davies CL, Budziszewska B, ***Sowa-Kućma M**, Opoka W, Reczyński W, Doboszewska U, Pilc A, Nowak G.: *Time course of zinc deprivation-induced alterations of mice behavior in the forced swim test.* Pharmacol Rep. 2012; 64:567-575. (IF 1.965; MNiSW 25)

Doboszewska U, ***Sowa-Kućma M**, Młyniec K, Pochwat B, Hołuj M, Ostachowicz B, Pilc A, Nowak G, Szewczyk B. : *Zinc deficiency in rats is associated with up-regulation of hippocampal*

NMDA receptor. Prog Neuropsychopharmacol Biol Psychiatry. 2015a;56:254-263. (IF 4.361; MNiSW 35)

Doboszewska U, Szewczyk B, *__Sowa-Kućma M__, Młyniec K, Ostachowicz B, Lankosz M, Nowak G.: *Antidepressant activity of fluoxetine in the zinc deficiency model in rats involves the NMDA receptor complex.* Behav Brain Res. 2015b;287:323-330. (IF 3.002; MNiSW 30)

3. The role of GPR39 receptor in the pathophysiology of depression

The metabotropic receptor GPR39 belongs to the ghrelin receptor subfamily and its natural agonist is zinc. The consequence of GPR39 stimulation is activation of the CREB transcription factor and as a result also BDNF synthesis. Due to the fact that the role of zinc in the pathogenesis of depression is undeniable, it seemed interesting to investigate also the role of GPR39 in the context of this disorder.

The study carried out in cooperation with the Jagiellonian University showed a significant reduction in the GPR39 protein level, accompanied by a decrease in BDNF level in mice after 6-week zinc deficiency diet. These alterations were correlated with increase in immobility time (pro-depressive behavior) in the forced swimming test, which confirms the importance of GPR39 receptor in the etiology of depression [Młyniec et al., 2013a]. Subsequent studies also showed a decrease in GPR39 receptor levels in the brain of rats fed a low-zinc diet and in *post-mortem* tissue of suicide victims. What's interesting, the oppostie changes were noted in the olfactory bulbectomy model of depression.

Młyniec K, Budziszewska B, Reczyński W, *__Sowa-Kućma M__, Nowak G.: *The role of the GPR39 receptor in zinc deficient-animal model of depression*. Behav Brain Res. 2013a; 238:30-35. (IF 3.391; MNiSW 30)

Mlyniec K, Doboszewska U, Szewczyk B, ***Sowa-Kućma M**, Misztak P, Piekoszewski W, Trela F, Ostachowicz B, Nowak G.: *The involvement of the GPR39-Zn(2+)-sensing receptor in the pathophysiology of depression and neurodegeneration. Part I. Study in rodent models and suicide victims.* Neuropharmacology. 2013b;79C:290-297. (IF 4.819; MNiSW 40)

4. The role of magnesium in the pathophysiology and treatment of depression

Magnesium is one of the most important elements in the human body, which participates in many basic physiological processes (e.g. it participates in energy metabolism as a cofactor of many enzymes and contributes to maintaining the fluidity of cell membranes, thereby exerting indirect modulating effects on neurotransmission). It is believed that the contribution of magnesium to the pathophysiology of depressive disorders is mainly related to its effects on the activity of N-methyl-D-aspartic (NMDA) receptors and γ-aminobutyric acid (GABA)-binding receptors. In addition, magnesium ions may have a modulating effect on the activity of other neurotransmission systems, including serotonergic, noradrenergic and dopaminergic systems.

Our case-control study, which enrolled 114 unipolar patients [Styczeń i wsp., 2015], 129 bipolar disorder patients [Siwek, et al., 2015] and 50 healthy volunteers [Styczeń i wsp., 2015; Siwek et al., 2015] showed a statistically significant increase in serum magnesium level in both groups of patients in the depressed chase, compared to the control group. Interestingly, in the remission, magnesium levels were not significantly different from the concentrations recorded in the health volunteers. These results may suggest a role of magnesium as a state marker reflecting the pathophysiological changes

underlying both unipolar and bipolar disorder and accompanying severe depressive episodes.

Styczeń K, Siwek M, ***Sowa-Kućma M**, Dudek D, Reczyński W, Szewczyk B, Misztak P, Topór-Mądry R, Opoka W, Nowak G.: *The serum magnesium concentration as a potential state marker in patients with unipolar affective disorder*. Psychiatr Pol. 2015;49(6):1265-76. (IF 0.884; MNiSW 15)

Siwek M, Styczeń K, ***Sowa-Kućma M**, Dudek D , Reczyński W, Szewczyk B, Misztak P, Opoka W, Topór-Mądry R, Nowak G.: *The serum concentration of magnesium as a potential state marker in patients with diagnosis of bipolar disorder.* Psychiatr Pol. 2015;49(6):1277-87. (IF 0.884; MNiSW 15)

Studies carried out in our laboratory using two animal models of depression: chronic mild stress (tha animals model of anhedonia) and olfactory bulbectomy (tha Animals model od agitated depression), indicated the antidepressant-like activity of magnesium, which was dependent on the NMDA/AMPA/BDNF cellular pathway.

Pochwat B, Szewczyk B, ***Sowa-Kucma M**, Siwek A, Doboszewska U, Piekoszewski W, Gruca P, Papp M, Nowak G.: *Antidepressant-like activity of magnesium in the chronic mild stress model in rats: alterations in the NMDA receptor subunits.* Int J Neuropsychopharmacol. 2014;17:393-405. (IF 4.009; MNiSW 40)

Pochwat B, ***Sowa-Kucma M**, Kotarska K, Misztak P, Nowak G, Szewczyk B.: *Antidepressant-like activity of magnesium in the olfactory bulbectomy model is associated with the AMPA/BDNF pathway.* Psychopharmacology (Berl). 2015;232:355-367 (IF 3.540; MNiSW 35)

5. Search for new treatment of depression

Due to the unsatisfactory efficacy of available pharmacological treatments for depressive disorders, there is a need to search for new antidepressants and study new strategies for potentiating and accelerating antidepressant treatment. One of the most interesting targets for new antidepressants are glutamatergic and serotoninergic receptors. On the other hand, the possibilities of potentiation of antidepressant therapy using atypical antipsychotics or dietary supplements seems equally interesting as well.

In the paper by Kłak et al. (2007), we focused our interests on (-)-N-phenyl-7-(hydroxyimino) cyclopropa[b]chromen-1a-carboxamide (PHCCC), which is a positive allosteric modulator of metabotropic glutamate receptor 4 (mGluR4). We examined the potential antidepressant-like activity of PHCCC after injection into the brain ventricles alone, or together with (1S,3R,4S)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid (ACPT-I), a nonselective group III mGlu receptor agonist, using the forced swimming test in rats. We found that ACPT-I induced a dose dependent antidepressant-like effect in FST, which was blocked by an antagonist of group III mGlu receptors (RS)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG). PHCCC injected intracerebroventricular was not effective, however when the compound was administered together with non-effective dose of ACPT-I, a profound antidepressant-like activity in FST was demonstrated. This effect was reversed by CPPG, group III mGlu receptors antagonist. These observations indicate that a combined administration positive allosteric modulation of mGlu4 receptor and agonists of group

III mGlu receptors may be a promising target in the future treatment of depressive disorder.

Kłak K, Pałucha A, Brański P**, *Sowa M,** Pilc A.: *Combined administration of PHCCC, a positive allosteric modulator of mGlu4 receptors and ACPT-I, mGlu III receptor agonist evokes antidepressant-like effects in rats.* Amino Acids. 2007;32:169-172. (IF 2.780; MNiSW 15)

In the paper by Siwek et al. (2009), the results of a double blind, placebo-controlled study conducted on 60 unipolar depressed patients fulfilling the DSM-IV criteria for major depression without psychotic symptoms were presented. Patients were randomized into two groups treated with imipramine (approximately 140 mg/day) and receiving once daily either placebo (n=30) or zinc supplementation (n=30, 25 mgZn/day) for 12 weeks. The obtained results indicate that zinc supplementation auguments the efficacy and speed of the onset of therapeutic response to imipramine treatment, especially in patients previously nonresponsive to antidepressant pharmacotherapies. These data also suggest the participation of disturbed zinc/glutamatergic transmission in the pathophysiology of drug resistance.

Siwek M, Dudek D, Paul IA, ***Sowa-Kućma M**, Zieba A, Popik P, Pilc A, Nowak G.: *Zinc supplementation augments efficacy of imipramine in treatment resistant patients: a double blind, placebo-controlled study.* J Affect Disord. 2009;118:187-195. (IF 3.763; MNiSW 24)

In the paper by Jastrzębska-Więsek et al. (2015) antidepressant-like properties of the compound EMD 38608, a partial agonist of the 5-HT6 receptor was demonstrated. The obtained results from behavioral studies in rats (including the forced swimming test and olfactory bulbectomy model) suggest that EMD 386088 produces antidepressant-like activity after systemic acute and chronic administration which may result from direct stimulation of 5-HT6 receptors.

Jastrzębska-Więsek , Siwek A, Partyka A, Szewczyk B, ***Sowa-Kućma M**, Wasik A, Kołaczkowski M,Wesołowska A.: *Antidepressant-like activity of EMD 386088, a 5-HT6 receptor partial agonist, following systemic acute and chronic administration to rats.* Naunyn-Schmiedeberg's Arch Pharmacol. 2015;388:1079-88. (IF 2.376; MNiSW 25)

B. *Cooperation with foreign and national scientific institutions*

1. Foreign institutions

- *Department of Psychiatry and Human Behavior, University of Mississippi Medical Centre, Jackson Mississippi – post-mortem* studies aimed at improving knowledge about the etiology of affective disorders
- *Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand* - clinical research on the role of oxidative stress and inflammation in the etiology of affective disorders; advanced statistical analysis

2. National institutions

- *Department of Affective Disorders, Chair of Psychiatry; Jagiellonian University Medical College, Kraków* – clinical research regarding the search for laboratory markers of affective disorders; creation of systematic reviews
- *Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków* – determination of zinc in biological samples
- *Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University, Kraków* – study of the distribution of metals in biological samples
- *AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Kraków* – determination of biometals in biological samples using advanced spectroscopic techniques
- *Department of Applied Pharmacy, Faculty of Pharmacy with Division of Medical Analytics Medical, University of Lublin* - study the role of the NMDA receptor in the pathophysiology and treatment of depression
- *Institute of Nuclear Physics Polish Academy of Sciences, Kraków* – analysis of biological samples using spectrometric techniques (Raman, FTIR, UV-vis spectroscopy)
- *Department of Forensic Toxicology, Institute of Forensic Research, Kraków* - acquiring human *post mortem* tissues (brain structures of suicide victims)
- *Department of Animal Physiology and Reproduction, Faculty of Biotechnology, University of Rzeszow; Centre of Applied Biotechnology and Basic Sciences* – protein analysis using immunofluorescence techniques
- *CNS Diseases Research Group, Celon Pharma, Łomianki* – cooperation/ consulting in the use of animal models in the search for new pharmacotherapy of psychiatric disorders

3. Membership in scientific organizations

- 2016 – present - 2nd Local Institutional Animal Care and Use Committee - **IACUC**
- 2016- present - Federation of European Neuroscience Societies – **FENS**
- 2015 – present – Polish Neuroscience Society - **PTBUN**
- 2014 – present –Polish Laboratory Animal Science Association - **PolLASA**
- 2007 – present - European Behavioural Pharmacology Society **- EBPS**

C. Participation in the research projects

1. Leadership

- **"The importance of interaction between NMDA and AMPA receptors and scaffolding proteins in the excitatory synaptic in the development and**

treatment of depression". (Funding: National Science Center; ID: UMO-2016/21/B/NZ7/01623; Implementation period: 14.02.2017 – 13.02.2020)

- "**Transcription factor MeCP2 as an intracellular object of study on the mechanism of depression and as a potential pharmacological target the disease".** (Funding: National Science Center; ID: UMO - 2013/09/D/NZ7/02520; Implementation period: 17.02.2014 – 16.02.2019)
- **"Allosteric modulation - a new strategy in pharmacotherapy. Identification of the psychotropic properties of group III glutamate receptor ligands".** (Funding: Co-financed by the European Union from the European Regional Development Fund; ID: UDA-POIG.01.03.01-12-100/08-00; Project leader: Prof. Andrzej Pilc, PhD) - **Task 8 manager**

2. Co-investigator

- **"Role of the nuclear transcription factor Nrf2 activation induced by (R, S) - Sulforaphane administration in the regulation of depressive behavior in the olfactory bulbectomy model in mice".** (Funding: National Science Center; ID: DEC-2016/23/N/NZ4/01337; Project Leader: Patrycja Pańczyszyn-Trzewik, M.Sc.; Implementation period: 13.09.2017-12.09.2020) - **Main Investigator**
- **"Effect of combined administration of fluoxetine and zinc in the chronic restraint stress in mice".** (Funding: National Science Center; ID: DEC-2016/21/N/NZ4/03252; Project Leader: Paulina Misztak, M.Sc.; Implementation period: 12.04.2017-11.04.2020) - **Main Investigator**
- **"Participation of cyclooxygenase-2 in the antidepressant action of group I metabotropic glutamate receptors ligands".** (Funding: National Science Center; ID: UMO-2014/13/D/NZ7/00292; Project leader: Katarzyna Stachowicz, Ph. D.; Implementation period: 12.02.2015-11.02.2019) - **Main Investigator**
- **"Evaluation of toxicity of selected antidepressants based on in vitro studies of rat spermatocytes, and mouse cell lines".** (Funding: National Science Center; ID: UMO-2014/15/N/NZ7/04097; Project leader: Przemysław Sołek, M.Sc.; Implementation period: 23.07.2015 – 22.07.2018) - **Scientific Supervisor**
- **"Innovative therapies for neurodegenerative and neurodevelopmental diseases based on allosteric modulators of mGlu receptors" (Allosterix)** (Funding: National Center for Research and Development; ID: PBS1/B7/ 8/2012; Project leader: Prof. Andrzej Pilc, Ph. D.; Implementation period: 2012-2017) - **Investigator**
- **"Depression - mechnisms - therapy".** (Funding: Co-financed by the European Union from the European Regional Development Fund; ID: POIG.01.01.02-12-004/09-00; Project Leader: Prof. Krzysztof Wędzony, Ph. D.) - **Main Investigator** (tasks: 3.2 and 3.3)

- **"Creation of a platform for seeking compounds acting on serotoninergic or glutamatergic systems as potential antidepressants or anxiolytics."** (co-financed from the Polish-Norwegian Research Fund; ID: PNRF-103-AI-1/07; project leader: Prof. Andrzej Pilc, Ph.D.; Implementation period: 2009-2011) - **Investigator**
- Internet Promotion of Science (Funding: co-financed by the EU, Priority: IV. Higher education and science. Operation: 4.2. Development of R&D system qualifications) - **Editor of the scientific notebook entitled "Biotechnology"**
- **"The role of zinc in adaptive mechanisms after antidepressants".** (Funding: Ministry of Science and Higher Education; ID: 2 P05A 0178 29; Project leader: Prof. Gabriel Nowak, Ph.D.; Implementation period: 2005-2008) - **Main investigator**
- **"Potential antidepressant properties of agonists of the III group of metabotropic glutamate receptors."** (Funding: Scientific Research Committee; ID: grant No. 3 P05A 077 25; Project leader: prof. Andrzej Pilc, Ph.D.; Implementation period: 2004-2006) **- Investigator**
- **"Can information on changes in light in the natural environment be transmitted via the humoral route from the retina to the central nervous system?"** (Funding: Ministry of Science and Higher Education; ID: N N 311 1001 33; Project leader: Prof. Marek Koziorowski, Ph.D.; Implementation period: 2007-2010) **- Investigator**

D. International and national awards

- **2018 - The ECNP CDE Grant** – conference scholarship (31th ECNP Congress, 6-9 October 2018, Barcelona, Spain)
- **2017 - The ECNP CDE Grant** - conference scholarship (30th ECNP Congress 2-5 September 2017, Paris, France)
- **2016 - The ECNP CDE Grant** - conference scholarship (29th ECNP Congress 17-20 September 2017, Vienna, Austria)
- **2016 - IBRO/FENS Young Investigator Training Programme 2016 -** scientific internship in Benedikz Group, Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark; Odense (http://cph2016.dk/2016/04/young-investigator-participants-announced/)
- **2014 - 2017** - annual prize in the "Qualitas" and "Quantitas" programs awarded by KNOW for works published in 2013-2016 by the employees of the Institute of Pharmacology, Polish Academy of Sciences in Krakow
- **2008 - The prize** of the Director of the Institute of Pharmacology, PAS **for the best publication of 2008**
- **2008** - **Doctoral dissertation with honors**, Institute of Pharmacology, PAS
- **2003-2007** - **Ph.D. Scholarship**, Institute of Pharmacology, PAS, Krakow, Poland

- **2007- EBPS Travel Award** (12th Biennal Meeting of EBPS, Tuebingen, Germany)
- **2004 r**. – **IBRO Travel grant** (The best student of International Brain Research Organization (IBRO) Summer School "Sensory and Integrative Neuroscience: From Receptors to Behavior", 18-31 August 2004, Moscow, Russia)

E. Oral presentations at conference and seminar

- *"Involvemant of 5-HT1 and 5-HT2 receptors in the antidepressant-like effect of zinc"* Sowa M, Kłak K, Nowak G.: 12th Biennal Meeting of EBPS, Tuebingen, Germany Behavioural Pharmacology, 2007, 18, Suppl.1, S75.
- *"The importance of interaction between NMDA receptors and scaffolding proteins in the development and treatment of depression"* Sowa-Kućma M: Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark; Odense, June 2016 - Benedikz Group seminar
- *„Increased oxidative stress in the frontal cortex of suicide victims is associated with changes in NMDA receptor, AMPK activity and magnesium level.*" Sowa-Kućma M, Panczyszyn-Trzewik P , Misztak P, Nowak G.: 31th ECNP Congress, 6-9 October 2018, Barcelona, Spain – poster Jam session

F. Scientific reviews

Until now, I have prepared **26 reviews of scientific articles** for international journals from Journal Citation Reports database, including: Neuro-Psychopharmacology & Biological Psychiatry; Psychiatry Research; PLOS One; Pharmacological Reports; Psychiatry and Clinical Neuroscience; Cellular and Molecular Neurobiology; Acta Neuropsychiatrica; International Journal of Experimental Pathology; Journal of Practical and Professional Nursing; Biological Trace Element Research; International Journal of Psychiatry and Clinical Practice; Journal of Human Nutrition and Dietetics

As an expert, I also reviewed scientific projects for the National Center for Research and Development and the Marshal Office of the Podkarpackie Region (Podkarpacki Scholarship Fund for PhD Students).

G. Stays at national and international scientific centers - participation in experiments

Jun/Jul 2016 - Benedikz Group, Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark – training in techniques used to study protein-protein interactions in biological samples

Feb/May 2016 – Faculty of Biotechnology, University of Rzeszów, Poland - training in the Immunofluorescence technique; protein analysis in rat brain sections after administration of antidepressants in various animal model of depression (part of the project UMO-2013/09/D/NZ7/02520)

Sep 2013 – Biomedical Education Center, Poznań, Poland – advanced training in molecular diagnostic techniques [Funding: Interdisciplinary PhD Studies project "Molecular sciences for medicine" (MOL-MED)]

May 2012 - Faculty of Chemistry, Jagiellonian University, Kraków, Poland - training in the MALDI-IMS (distribution of zinc in rat brain sections)

Jan/Nov 2007 - Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków, Poland - training in the differential pulse anodic stripping voltammetry (DPASV); determination of zinc in rat brain mikrodialysate using DPASV (part of the project no. 2 P05A 0178 29)

H. Teaching achievements and science popularization

1. Activities and teaching achievements

2017 - present Medical Faculty, University of Rzeszów

- Human Physiology course for medical students – coordinator and lecturer (both laboratory classes, seminars and lectures)
- Student Scientific Society of Physiology "NEURON" - supervisor

2004 – September 2018 Institute of Pharmacology Polish Academy of Sciences, Kraków

- Training of M.Sc. and Ph.D. students, as well as trainees in the field of animal studies and biochemical techniques

- Supervisor of 4 Master's theses implemented by M.Sc. students of pharmacy and neurbiology

- **Auxiliary supervisor of 2 Ph.D. students**: Paulina Misztak (Ph.D. student at Jagiellonian University Medical College; thesis entitled: "The role of the transcription factor MeCP2 in depression"; opening data: 12 Sep 2017) Patrycja Pańczyszyn-Trzewik (Ph.D. student at the IP PAS; thesis entitled: "The importance of inflammation and oxidative stress in the pathogenesis of depression – study the role of the nuclear transcription factor Nrf2; opening date: 16 Oct 2018). Both Ph.D. students are managers of research projects (Prelude 11 and 12, respectively) financed by the National Science Center.

- Paulina Misztak is a beneficiary of the Scholarship of the Minister of Science and Higher Education for Ph.D. students for outstanding achievements in the academic year 2016/2017.
  Both Ph.D. students are multiple beneficiaries of mobility scholarships awarded by international scientific organizations and committees (including: FENS/IBRO and ECNP). Morover, Patrycja Pańczyszyn-Trzewik received

several times the award for the best poster presentation at national and international conferences.

2008-2015 Faculty of Biotechnology, University of Rzeszów

- Different courses for biotechnology students, including:
    - Molecular biology (course cooridnator; lectures and laboratory classes)
    - Molecular biology of prokaryotic and eukaryotic organisms (lectures)
    - Medical biotechnology (course cooridnator; lectures and laboratory classes)
    - Genetic markers (laboratory classes)
    - Protein markers (laboratory classes)
    - Cell biochemistry (lectures)
    - Molecular biology in diagnostics of diseases (laboratory classes)
    - Methodology and optimization of research techniques (laboratory classes)
    - "RNA analysis techniques" within the project: Biotechnology students as an accelerator of a Knowledge-Based Economy financed from the funds of the Human Capital Operational Program - POKL.04.01.02.00-038 / 09-00 (course coordinator - development of a course program; laboratory classes)
    - "Molecular Pharmacology" within the project: Biotechnology students as an accelerator of a Knowledge-based Economy financed from the funds of the Human Capital Operational Program - POKL.04.01.02.00-038 / 09-00 (course coordinator - development of a course program; laboratory classes)

- Lectures (courses: Pharmacology, Pharmacognosy, Phytotherapy) for post-graduate students of the University of Rzeszów (academic year 2014/2015)

- Supervisor of 21 B.Sc. and 22 M.Sc. students. Seven of them were awarded an Erasmus Scholarship for long-term internship abroad. One of them (Piotr Pankiewicz, M.Sc.) has also been awarded the Scholarship of the Minister of Science and Higher Education for M.Sc. students for outstanding achievements in academic years 2012/2013.

- **Auxiliary supervisor of Ph.D. student**: Przemysław Sołek (thesis entitled: "The use of a cellular model for *in vitro* studies of the mechanisms of antidepressants' toxicity with a different side effects"; opening data: 27 Jun 2018)

2. Science popularization

- **Editor of the scientific notebook entitled "Biotechnology"** within the project: Internet Promotion of Science (co-financed by the EU, Priority: IV. Higher education and science. Operation: 4.2. Development of R&D system

development) – The main goal of this publication was to popularize the achievements of biotechnology and their significance for modern society.

- **Co-authorship of the chapter "Zinc Deficiency and Depression"** (Rafalo A, Sowa-Kucma M, Pochwat B, Nowak G, Szewczyk B) in "Nutritional Deficiency" edited by Pınar Erkekoglu and Belma Kocer-Gumusel, ISBN 978-953-51-2438-2. The main purpose of this book is to determine the relationship between the state of nutrition and general health. The aim of the chapter is to discuss the importance of zinc in the etiology and treatment of depression.
- **Scientific supervisor of the Student Scientific Society of Physiology (SSSP) "NEURON"** – the main objective of the SSSP is to promowe scientific research and popularization of science among medical students of University of Rzeszow
- **Expert in the "Radio Center of Science"** – the broadcast of Radio Rzeszów - the program is focused on inventions and research that has changed the world; the program also explains to the radio listeners the principles of the functioning of the human body

I. Organizational activity

- Aug 2017 - Head of the **Laboratory of Innovative Research on Circulatory and Respiratory Systems**; Centre for Innovative Research in Medical and Natural Sciences, University of Rzeszow
- Oct 2017 – **Head of the Department of Human Physiology**, Medical Faculty, University of Rzeszow
- Oct 2017 - **Coordinator of Human Physiology course** for medical students, Medical Faculty, University of Rzeszow