Charles University

Faculty of Science

Study programme: Biology

Branch of study: Biology

Ilia Shalkin

Posttraumatická stresová porucha a její biomarkery

Post-traumatic stress disorder and its biomarkers

Bachelor´s thesis

Supervisor: Mgr. Petr Telenský, Ph.D.

Prague, 2021

Prohlášení:

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V Praze, 13.08.2021

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Ilia Shalkin

Acknowledgements

I would like to thank my supervisor, prof. Petr Telensky, Ph.D., for his time and valuable advice. I desire to thank my family and friends for their support.

Abstract

Post-traumatic stress disorder (PTSD) originally referred to conditions observed among military people. It was first widely accepted as a diagnosis during the First World War. As a result, PTSD was no longer attributed exclusively to the military environment and, at the moment, there are a large number of studies that relate to civilians who developed trauma as a result of a terrorist attack, rape, the death of a loved one, natural and man-made disasters.

Severe trauma can affect everyone, and most people manage to cope with stress and continue to live their everyday lives. However, some people may develop PTSD. A person diagnosed with PTSD may have sleep disorders and nightmares, increased irritability, feel guilty and look for the cause of problems in themselves, and may not experience positive emotions. Symptoms of PTSD have a destructive effect on the patient himself and affect loved ones, leading to a break in social ties and loss of work. PTSD is a complex disease affecting various regulatory systems of the body, but despite many studies, the aetiology of PTSD development is not clear today. This fact limits scientists in developing a treatment for PTSD.

One of the ways to develop research on the treatment of PTSD uses preclinical and clinical methods to track biomarkers associated with changes in the body after injury. The study of biomarkers will bring us closer to understanding the aetiology of PTSD, direct further pharmacological research for drug development, and allow us to identify risk groups of people, preventing the development of PTSD.

This thesis examines biomarkers and their research methods that can potentially help to understand the causes of PTSD. Scientists look at disorders from all possible angles, examining hundreds of different biomarkers. The format of this paper does not allow us to describe all the processes that occur in PTSD, so the paper describes the main biomarkers studied and methods for their study to get closer to understanding the processes behind PTSD.

The introductory part of the paper discusses the definition and impact of stress on the human body. For understanding the physiological processes that occur in response to stressors, the HPA axis is considered, followed by a description of the “Two-Hit” hypothesis, as a possible theory of the origin of PTSD. The second chapter examines the current definition of PTSD and the risk factors that contribute to the development of PTSD. The third chapter provides examples of preclinical and clinical methods for studying biomarkers related to memory and regulatory disorders. The fourth chapter describes the biomarkers studied. The fifth chapter describes the problems of research and conclusions.

Keywords: Post-traumatic Stress Disorder, Biomarker, Preclinical Method, Clinical Method, Stress, “Two-hit” hypothesis, Allostatic Load

Abstrakt

Posttraumatická stresová porucha (PTSP) původně patřila mezi poruchy pozorované mezi vojáky. Do širšího povědomí veřejnosti se tato porucha dostala jako diagnóza během první světové války. Následně už nebyl PTSP přisuzován výlučně vojenskému prostředí a v současné době existuje velké množství studií, které se týkají civilistů, u nichž došlo k traumatu v důsledku teroristického útoku, znásilnění, smrti milované osoby nebo přírodních a antropogenních katastrof.

Každý člověk může být vážně traumatizován a většina lidí se vyrovnává se stresem a pokračuje v normálním životě. U některých lidí se však může vyvinout PTSP. Osoba s diagnostikovanou PTSP může mít poruchy spánku a noční můry, zvýšenou podrážděnost, cítit se provinile a hledat příčinu problémů uvnitř sebe a ztratit schopnost prožívat pozitivní emoce. Příznaky PTSP mají destruktivní účinek nejen na pacienty s touto poruchou, ale postihují také jejich blízké, což vede k rozpadu sociálních vazeb a ztrátě práce. PTSP je komplexní onemocnění postihující různé regulační systémy těla, nicméně navzdory velkému počtu studií není dnes etiologie vývoje PTSP jasná. Tato skutečnost omezuje vědce ve vývoji léčby PTSP.

Jednou z cest pro rozvoj výzkumu léčby PTSP je použití preklinických a klinických metod pro sledování biomarkerů spojených se změnami v organismu po traumatu. Výzkum biomarkerů může přivést nás blíže k porozumění etiologii PTSP, usměrnit další farmakologický výzkum vývoje léčiv a umožnit identifikaci rizikových skupin populace k prevenci PTSP.

Tato práce zkoumá biomarkery a metody jejich výzkumu, které mohou potenciálně pomoci porozumět příčinám vzniku PTSP. Ve vědecké literatuře je tato porucha probádána z více stran a pozornost vědecké komunity byla věnována velkému množství biomarkerů. Rozsah této práce neumožňuje popsat všechny procesy vyskytující se v PTSP, proto v práci jsou uvedeny hlavní biomarkery a metody jejich výzkumu, které by mohly pomoci porozumět procesům spojeným s PTSP.

Úvodní část práce zkoumá definici stresu a jeho vliv na lidský organismus Abychom porozuměli fyziologickým procesům, ke kterým dochází v reakci na stresory, je předvedena osa HPA, následně je popsána „Two-Hit“ hypotéza jako možná teorie původu PTSP. Druhá kapitola se zabývá současnou definicí PTSP a rizikovými faktory, které přispívají k rozvoji PTSP. Třetí kapitola uvádí příklady preklinických a klinických metod studia biomarkerů spojených s paměti a poruchami regulačních mechanismů. V čtvrté kapitole jsou popsány studované biomarkery. Pátá kapitola obsahuje problematiku výzkumu a závěr.

Klíčová slova: Posttraumatická Stresová Porucha, Biomarker, Předklinické Metody, Klinické Metody, Stres, “Two-hit” hypotéza, Alostatická Zátěž

List of Abbreviations

ACC – Anterior cingulate cortex

BDNF – Brain-derived neurotrophic factor

CT – Computed tomography

DSM-5 - Diagnostic and statistical manual of mental disorders, 5th edition

ECG – Electrocardiogram

EDA – Electrodermal activity

ELISA – Enzyme-linked immunosorbent assay

fMRI – Functional magnetic resonance imaging

GWAS – Genom-wide association study

HPA – Hypothalamus-pituitary-adrenal axis

HR – Heart rate

HRV – Heart rate variability

IBI – Interbeat interval

MRI – Magnetic resonance imaging

PFC – Prefrontal cortex

PTSD (PTSP) – post-traumatic stress disorder (posttraumatická stresová porucha)

PPS – Predator-based psychosocial stress

SPECT – Single-photon emission computed tomography

vmPFC – Ventromedial prefrontal cortex

Contents

1. Introduction

1.2 Stress and Allostatic load

Stress factors are any factors that bring the body out of homeostasis. The stress response, thanks to physiological mechanisms, allows us to return to homeostasis. The Fight-or-Flight response allows us to avoid danger and survive (Walter B. Cannon, 1915).

The term "stress" was first coined by American physiologist Walter Cannon (Walter B. Cannon, 1932) in 1932. At the time of publication, this term did not have the definition that it has today since it meant neuropsychic stress. One of the modern definitions of "stress" belongs to the endocrinologist Hans Selye (Selye, 1946), known for the formulation of the General Adaptation Syndrome, where "stress" means a non-specific adaptive response to the impact of stressful factors.

The stress response triggers the mechanism of adaptation for the optimal condition under new conditions by mobilizing the body's resources to deal with stress. The stress response in the human body is associated with producing endogenous chemicals such as cortisol, epinephrine, norepinephrine, and dopamine. Usually, short-term stress does not harm the body, and the above substances help the body cope with stress (Selye, 1975).

Allostatic load is the wear and tear of the body that accumulates when exposed to one or more stressors for a long time. Chronic stress can lead to an overproduction of chemicals and disruption of the stress response shutdown mechanisms. At the same time, prolonged exposure of stress on the body exhausts it, the process of returning homeostasis inhibits other body systems. This type of influence can cause psychological illnesses (depression (Bay *et al.*, 2002), PTSD, sleep disorders (da Estrela *et al.*, 2021), bipolar disorder (Ostiguy *et al.*, 2009), immune system depression (Ulmer-Yaniv *et al.*, 2018), and many other problems with the body that will be discussed later in this paper.

1.3 Stress in affective disorders

Depressive, bipolar, panic, anxiety, and other affective disorders are understood primarily as stress disorders. The fundamental neural circuits that regulate stress reactivity do not function optimally in these disorders. Among the consequences of deregulation, the increased reactivity to stressors and the inability to extinguish the stress response is particularly pronounced. Also, physiological factors that affect the body's stress transfer include increased levels of circulating glucocorticoids, sleep and wake disorders, jet lag, and inactivity (McGinn and Pahng, 2017).

1.4 The role of the hypothalamic-pituitary-adrenal axis (HPA)

When transmitting a signal about stress, the signal is processed in the amygdala, responsible for fear conditioning and regulating emotional response (Hrybouski *et al.*, 2016). The hippocampus also processes the signal, which consolidates memory and participates in comparative analysis with information about past stresses (Stevens *et al.*, 2018). If stress has been successfully experienced before, then the hippocampus can inhibit the stress response. However, if the stress response continues, the signal activates the HPA axis, a key mechanism in the stress response. The HPA axis consists of the hypothalamus, pituitary gland, and adrenal glands. First of all, the hypothalamus secretes corticoliberin, which enters the pituitary gland. Then, the pituitary gland reacts by releasing adrenocorticotropic hormone, which binds to adrenal receptors through the blood, leading to the release of catecholamines, mineralocorticoids and glucocorticoids.

1.5 "Two-Hit" hypothesis

The modern scientific community is well aware that stress affects human health, especially at a young age. This idea is best supported by the "Two-Hit" hypothesis, which shows us a combination of factors that lead to diseases.

This hypothesis explains the development of the disease in the case of a combination of two stages. The first stage "First Hit" is a predisposition to the disease that does not develop. However, with the onset of the second stage "Second Hit", psychopathology develops, associated with the stress experienced (Knudson Jr, 1971).

1.6 Importance of biomarkers

World Health Organisation defines biomarkers as: "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction." (WHO, 1993).

Identifying various biomarkers contributes to prevention and prevention in high-risk groups, such as people in the military. In addition, biomarkers can develop the best treatment option and identify the risk of developing diseases (Zhou *et al.*, 2012).

Clinical biomarkers should be investigated and tested according to certain principles. Biomarkers must meet criteria for analytical validity (is testing accurate?), clinical validity (are the results relevant to medicine?), clinical benefit (how will the study help improve health care?), as well as several other criteria, such as psychological and ethical implications, cost-effectiveness, and the future benefits that the research team implements based on the results. Biomarkers are slow to enter

clinical use, as their discovery takes a significant amount of time. Often, even after the discovery, the biomarker does not quickly become meaningful in its use (in review Selleck, Senthil and Wall, 2017).

2. Post-traumatic stress disorder (PTSD)

2.1 Definition and diagnostic criteria

Post-traumatic stress disorder (PTSD) refers to a group of psychological disorders associated with stress. PTSD is an integrated complex of symptoms of mental disorders that have arisen as a result of a single or repeated external super-strong traumatic impact on the human psyche (physical and/or sexual violence, the constant nervous strain associated with fear, humiliation, empathy for the suffering of others, military operations, natural disasters and other things) (American Psychiatric Association, 2013).

Symptoms of PTSD may not appear immediately and may not manifest for a long time after a traumatic event (American Psychiatric Association, 2013).

Post-traumatic stress disorder is characterized by a state of increased anxiety, against the background of which painful memories occasionally arise when encountering triggers (keys), which are stimuli that are a fragment of the memory of a traumatic event (a child crying, screeching brakes, the smell of gasoline, the hum of a flying plane). PTSD is characterized by partial amnesia so that the patient cannot remember all the details of the traumatic situation. Symptoms of PTSD may not appear immediately and may not manifest themselves for a long time after a traumatic event. The main symptoms may appear immediately in the first days and after six months (American Psychiatric Association, 2013).

Due to constant nervous strain and characteristic sleep disorders (nightmares, insomnia), over time, patients with PTSD develop the so-called cerebrastenic syndrome (a complex of symptoms indicating the exhaustion of the central nervous system), as well as disorders of the cardiovascular, endocrine, digestive and other leading systems of the body (World Health Organization, 2004).

2.2 Criteria for PTSD

The criteria for PTSD can be divided into two systems currently used globally - the ICD-10 system created by the WHO and the DSM-5 system created by the American Psychiatric Association.

Most often, PTSD is associated with a constant obsessive recall of situations associated with a stressor. The situation of obsessive dreams is also widespread, where the moment of stress is experienced again. However, this alone is not enough to diagnose PTSD. The person must survive the traumatic event and have a long-term combination of various symptoms beyond obsessive recall, including avoiding circumstances that resemble or are associated with the stressor (American Psychiatric Association, 2013).

ICD-10 has an indirect definition of complex PTSD, characterized by dissociation, sudden mood swings, feelings of detachment, and negative emotions (Shevlin *et al.*, 2018).
Table 1 shows the DSM-5 criteria for monitoring and diagnosis of PTSD.

Table 1. DSM-5 criteria for PTSD. *From* (American Psychiatric Association, 2013).

DSM-5 criteria for PTSD

Trauma exposure	
Trauma	Actual or threatened violent death, serious injury or accident, or sexual violence
A. Exposure	Via any of the following: 1. Directly exposed to trauma 2. Eyewitness (in person) to others directly exposed to trauma 3. Learning of direct exposure to trauma of a close family member or close friend 4. Repeated or extreme exposure to aversive details of traumatic event (eg, trauma workers viewing human remains or repeatedly exposed to details of child abuse), in person or via work-related electronic media
Symptom groups B to E (symptoms beginning or worsening after the traumatic event)	
B. Intrusion	*≥1 intrusion symptoms:* 1. Recurrent, involuntary, distressing trauma memories 2. Recurrent, distressing trauma-related dreams 3. Dissociative reactions/flashbacks related to trauma 4. Intense or prolonged psychological distress to trauma reminders 5. Marked physiological reactions to trauma reminders
C. Avoidance	*≥1 avoidance symptoms:* 1. Avoidance/efforts to avoid distressing internal trauma reminders (memories, thoughts, feelings) 2. Avoidance or efforts to avoid distressing external trauma reminders (people, places, activities)
D. Negative cognition and mood	*≥2 negative cognition/mood symptoms:* 1. Amnesia for important parts of trauma exposure 2. Persistent, exaggerated negative beliefs about self, others, or the world 3. Persistent, distorted trauma-related cognitions leading to inappropriate blame of self/others 4. Persistent negative emotional state (eg, fear, horror, anger, guilt, shame) 5. Loss of interest or participation in significant activities 6. Detached/estranged feelings from others 7. Persistent loss of positive emotions (eg, happiness, satisfaction, love)
E. Hyperarousal	*≥2 marked alterations in trauma-related arousal and reactivity:* 1. Irritability and angry outbursts with little/no provocation (eg, verbal/physical aggression toward people/objects) 2. Reckless or self-destructive behavior 3. Hypervigilance 4. Exaggerated startle 5. Concentration problems 6. Sleep disturbance (eg, difficulty falling or staying asleep, restless sleep)
Additional criteria	
F. Duration	>1 month
G. Distress/ impairment	Clinically significant distress; social/occupational/other important functioning impairment
H. Not attributable to another disorder	Independent of physiological effects of a substance (eg, medication, alcohol) or another medical condition

2.3 Risk factors

Most people experience a major traumatic event throughout their lives. However, most manage to cope with the trauma and return to normal. However, some people develop PTSD, and several factors increase their risk of developing PTSD.

There is a risk group whose representatives are most susceptible to the occurrence of PTSD syndrome. These include medical service workers, rescuers, participants in extreme events themselves and their family members (American Psychiatric Association, 2013).

There are individual characteristics that increase the risk of developing PTSD, such as heredity weakness (mental illnesses, suicides, alcohol, drug or other types of addiction in close relatives) (Shalev *et al.*, 2019); psychological trauma suffered in childhood (Bomyea *et al.*, 2020); concomitant nervous, mental or endocrine diseases, hormone levels during puberty, which play an essential role in brain ontogenesis; social loneliness (lack of family, close friends); difficult economic situation (in review Bryant, 2018). In addition, research shows that traumatic experiences, such as sexual abuse, affect brain development and disrupt major hormonal systems (Carrion *et al.*, 2009).

One of the most important risk factors is gender and age. Women are twice as likely to suffer from PTSD. It is also affected by the greater likelihood of experiencing interpersonal violence (rape, sexual harassment, lack of parental control) (Forbes *et al.*, 2014).

Genetics also plays an essential role among risk factors. For example, genetics determines the presence or absence of psychological illnesses, and genetics determines a person's ability to withstand stress and stressful factors (Stevens *et al.*, 2013). Recent studies of the interaction of genes and the environment (G × E) have revealed a pattern in which most of the variance for this result is explained not only by the influence of genotype or phenotype but also by the influence of an individual's life experience (Rutter, Caspi and Moffitt, 2003).

3. Methods of study and identification of PTSD biomarkers

3.2 Preclinical methods

One of the most critical research approaches is preclinical models that complement clinical studies. They can significantly improve our understanding of the neurobiological underpinnings of the

problem we are considering. This thesis is focused on animal models since most preclinical experiments related to PTSD use animal models.

Using simulations in laboratory animals to produce PTSD-like symptoms designed for use in psychiatry and experimental medicine. A wide variety of studies have examined many different stressors for recreating PTSD in animal experiments. For example, physical, psychosocial, and metabolic problems (in monography Stam, 2007). In addition, electric shock (Wakizono *et al.*, 2007), underwater trauma (Cohen, Liberzon and Richter-Levin, 2009), stress-restress (Zoladz *et al.*, 2015), and single long-term stress paradigms were used (Perrine *et al.*, 2016), as well as exposure to predators or signals that are associated with predators (Zoladz *et al.*, 2015). The main symptoms caused are a violation of social activity (Nelson, Demartini and Heinrichs, 2010), increased fear in connection with a specific stressor (Zoladz, Fleshner and Diamond, 2012), and cognitive impairment (Wang *et al.*, 2010).

For the validity of animal studies and interpretation of the data obtained, researchers need to demonstrate observable and measurable behavioural responses during the simulation of clinical symptoms. Ideally, the animal model should fully repeat the symptoms of PTSD (facial validity), have a theoretical justification for the processes occurring (validity of the study mechanics) and a satisfactory response to treatment, ideally identical to what we can theoretically observe in humans (validity of the prognosis) (in review Borghans and Homberg, 2015).

3.2.1 Physical stressors

When using physical stressors, we work with aversive stimuli to recreate the stress of the subjects. The main challenge is to recreate near-death experiences or accidents, such as those experienced by soldiers and rescuers. These stressors benefit from their procedural simplicity, precise exposure to symptoms, and ease of scaling. Now we will look at some of the research models that are most often used in trials.

This paradigm includes various stressors, such as immobilization, forced swimming, and exposure to ether delivered simultaneously to the subject. It is used to study non-naturalistic, uncontrolled situations that can trigger maladaptive fear responses in many anxiety and traumatic disorders.

3.2.1.1 Single prolonged stress

The first considered method involves experimental animals and simultaneous contact with a stressor to develop maladaptive fear reactions. According to the standard, rats are subjected to severe combined stress, including 2 hours of immobilization, 20 minutes of forced swimming, and 15

minutes of ether stress until they lose consciousness. This model is used to diagnose hormonal symptoms of PTSD in laboratory animal models. Then, hormone tests are periodically diagnosed. In each sample, the level of corticosterone is determined (Tanaka *et al.*, 2018). When it differs between concentrations in the samples, the method is used in further PTSD studies and traumatic disorders (Knox *et al.*, 2012).

3.2.1.2 Stress enhanced fear learning

This method uses an electric shock to study the extinction of fear that occurs in patients with PTSD. According to studies, the weekly return of animals to a shocking context increases their acoustic response of fright, indicating hyperexcitation. The stress enhanced fear learning group also includes the underwater trauma method, which is used, for example, for Intrusive re-experiencing of a symptom (Li *et al.*, 2021). This method differs from the forced swim test in the depth of the test vessel and the depth of immersion of the subject. In these studies, the rats are submerged for 30 seconds underwater to a depth of 30 cm (Yankelevitch-Yahav *et al.*, 2015).

3.2.2 Social and psychological stressors

Dealing with social stressors is another type of challenge for recreating stress and PTSD. However, instead of physical harm, most often, to recreate social stress, the usual social environment and the usual parts of life for the animal world are taken – the hierarchy, the society in which the animal lives, the area of its habitat.

3.2.2.1 Predator based psychological method

Predator-based psychosocial stress (PPS) suggests a more naturalistic approach to fear training. The animal is periodically immobilized during contact with a natural predator, such as a cat or snake. With no need in a live predator animal, a more convenient method for research is the predator scent stress method, which uses the smells of natural predators. Rats come into contact with the excrement of a predator animal, resulting in stress (Cohen *et al.*, 2007). It leads to increased levels of anxiety, impaired cognitive function, cardiovascular reactivity, and startle response (Long and Fanselow, 2012).

3.2.2.2 Housing instability

According to this model, individual animals are randomly moved to different cages (Zoladz *et al.*, 2008). When this model is used, the symptoms of PTSD are recreated by isolating the individual from the social environment. Moreover, it is often taken in combination with the predator psychological stress. After this combined procedure, the mice showed poor acclimatization to new

conditions (Saavedra-Rodríguez and Feig, 2013). Effects found in rats include increased corticosterone suppression, HPA axis dysfunction, and increased freezing to a stressful context (Zoladz, Fleshner and Diamond, 2012).

3.2.2.3 Social defeat

This method simulates interpersonal violence. The research group is also able to recreate stressful conditions directly in the studied society. For example, in the model, subjects are pressured by a more dominant member of their species to attack an intruder. Usually, within four days, the intruder is placed in a cage with the dominant animal for 15 minutes, and the next day the corticosterone level is measured (Yang *et al.*, 2013).

Depressed animals can be classified as receptive or resistant, and although both express anxious behaviour, only the receptive population shows increased avoidance (Tse *et al.*, 2014).

3.2.2.4 Early life stress

The method is used to recreate childhood trauma. Reproducing social instability through maternal isolation in rats shows the same results as the social isolation model in adult animals. Maternal animal separation simulates childhood trauma, usually from 2 to 9 days after birth, separating the mother and puppies for one or several hours (Liu *et al.*, 2017). When combined with the single prolonged stress model in adulthood, separation from the mother reinforces anxious behaviour and behaviours associated with contextual freezing. Animals exposed to stressors at an early age are more likely to develop symptoms of extreme anxiety when stressed in adulthood (Imanaka, 2006).

3.3 Clinical methods

After understanding what preclinical trials are and how they are conducted, the logical next step is to discuss clinical trials conducted in humans. In general practice, the basic rules for conducting such tests meet the classical postulates: it is necessary to have a research protocol and its theoretical justification, proven in preclinical studies, it is necessary to have competent personnel, as well as the availability of all means of protection for those who are subjected to research. In addition, of course, it is also necessary to comply with legal conventions – to obtain regulatory approval, as well as to comply with the legal and ethical legitimacy of research and to obtain voluntary consent from each subject (in monography Sean Tunis, Allan Korn, 2002).

Clinical trials, just like preclinical ones, have in their arsenal some methods by which they function. Therefore, to understand the topic of clinical trials, one needs to familiarize oneself with these methods.

3.3.1 Methods of researching genes biomarkers

The study of genes is necessary to identify specific biological mechanisms that underlie the development of PTSD. Therefore, to understand clinical research, it is necessary to focus on the method of searching for candidate genes, and candidate genes with consideration of the environment (cGxE) since this method plays a significant role in the study of PTSD.

Candidate gene studies evaluate the main effects of gene expression. In these studies, candidate genes are selected based on the criteria of the biological effect of the gene on the trait or disease under consideration, based on existing data on the pathophysiology of PTSD (Hoxha *et al.*, 2019). Research focuses on detecting single-nucleotide polymorphisms, for example, using the polymerase chain reaction method (Guo *et al.*, 2018).

The most verifiable candidate genes here are those involved in the regulation of brain-derived neurotrophic factor (BDNF) (Jin *et al.*, 2019), serotonergic (SLC6A4) (Wang *et al.*, 2011), dopaminergic (SLC6A3) (Chang *et al.*, 2012), and the HPA axis system (FKBP5) (Jaksic *et al.*, 2019).

Candidate gene-environment interaction (cGxE) studies are a natural extension of the candidate gene design. This method, as mentioned above, examines both genetic influence and environmental variables. These studies have become a logical extension of candidate genes' design because often, the disease itself may not be inherited (Uddin *et al.*, 2013). Instead, offspring are passed on disease risk factors related to the individual's environment. From this thought, we can understand why people with different genotypes suffer differently from the same environmental factors. The interaction of an individual's unique genes and environmental factors leads to the formation of different phenotypes of the disease (Kimbrel *et al.*, 2016).

Genome-wide association study (GWAS) does not use preliminary hypotheses about the role of specific genes and their loci. Instead, in the course of a single analysis, up to one million variants of single-nucleotide polymorphisms are studied in a large group of individuals (Logue *et al.*, 2013). Thus, the GWAS method does not necessarily determine clear links between genes and phenotypic manifestations. However, the broad coverage of the studied genes potentially allows one to find links with causal genes with weak expression (Duncan *et al.*, 2019).

3.3.2 Methods of researching hormones biomarkers

Various hormone testing methods help determine hormone levels in the body. Researchers take for analysis urine (Delahanty *et al.*, 2005), saliva (Freidenberg *et al.*, 2010), hair (Pacella *et al.*, 2017) and blood test (Jergović *et al.*, 2015). It is essential to take into account significant changes in

hormone levels throughout the day in order not to lose the validity of studies. For example, cortisol has elevated levels in the morning (Wessa *et al.*, 2006).

The radioimmune analysis uses the reaction of radionuclide-labelled antigen or antibodies. This method allows one to study the concentration of cortisol in saliva, which reflects the concentration of cortisol in the blood (Boks *et al.*, 2016). There is also a modern method, such as an enzyme-linked immunosorbent assay (ELISA) (Malone *et al.*, 2001), which also uses the antigen-antibody reaction, and mass spectrometry (Karabatsiakis *et al.*, 2015).

3.3.3 Neurovisualisation

This category of methods is used to study structural changes in the brain. Analysis of changes in brain structure is performed using magnetic resonance imaging (MRI) (Richert *et al.*, 2006), magnetic encephalography (MEG) (Huang *et al.*, 2014), computed tomography (CT) (Abrams *et al.*, 2013), and Single-photon emission computed tomography (SPECT) (Seedat *et al.*, 2004).

Studying brain structures changes allows us to understand better the aetiology of PTSD in the study of areas related to stress response and memory, especially the amygdala, hippocampus, and PFC (Shi and Davis, 2001).

3.3.4 Neuropsychological methods

3.3.4.1 Electroencephalograph (EEG)

It is a method of recording brain electrical activity recorded through intact skin and cranial tissues. Unlike the neuroimaging methods described earlier, this method is characterized by a high time resolution of potential removal, with an accuracy of up to milliseconds. A disadvantage of EEG measures compared to functional brain imaging techniques such as fMRI is relatively poor spatial resolution. Examples of measurable EEG parameters are spontaneous EEG activity, evoked potentials (distinct patterns of EEG signal change following a stimulus), EEG coherence (measures of similarity in signals recorded from distinct channels, typically used as a measure of functional connectivity), or spectral power analyses (Wang *et al.*, 2020).

Measuring signals from the cerebral cortex in patients with PTSD can help study changes in frequency ranges to identify dysfunctions of parts of the cerebral cortex (Sponheim *et al.*, 2011).

3.3.4.2 Electrocardiogram (ECG)

Momentary changes in emotional states and long-term emotional well-being are associated with heart activity changes, namely heart rate (HR) and heart rate variability (HRV). The heart rate is

visible on cardiograms, measured in beats per minute or interbeat intervals (IBI). HRV refers to small variances in IBIs of the heart. HRV parameters are typically obtained by computer processing of raw ECG data. Measures of HRV can be presented either as statistical measures of variances in IBIs (time-domain HRV metrics, e.g. SDRR – Standard Deviation of RR intervals or RMSSD – Root mean square of successive differences in RR intervals) or as frequency-domain metrics based on Fourier transform or autoregressive analyses (e.g. LF/HF – a ratio of low-frequency and high-frequency power).

These indicators can also be tracked in a non-invasive way, using electrodes placed on the body. The ECG method allows one to record disorders caused by chronic stress and show how the body reacts to stressful conditions. Research results have shown that HR decreases in the absence of fear and increase if there is a fear-eliciting stimulus. HR also increased when the conditioned stimulus was applied. A recent study found that longer-term HRV measures can be used to indicate emotional dysregulation in trauma-exposed individuals, although immediate HR responses to acute stressors did not differ in traumatized and healthy adults (Powers *et al.*, 2021).

3.3.4.3 Electormyography (EMG)

EMG testing helps assess the electrical activity of skeletal muscles and related nerve functions. For research on PTSD, this is useful, as scientists use this technology to measure the contractions of facial muscles that underlie the expression of emotions (Cacioppo *et al.*, 1986). Of course, even the most minor muscle groups are subjected to a person's volitional effort, which, at first glance, may indicate the bias of the method. However, researchers rely primarily on fast reactions, for example, a fast startle reaction (it has a latency of 20-200 ms), which cannot be performed intentionally (Seligowski *et al.*, 2016). To recreate such reactions, researchers use sources of stress, such as an acoustic stimulus in the form of short bursts of white noise, which is fed to the subject through headphones. Provoked reactions are defined as an increase in the frequency of the acoustic startle response in the presence of a previously neutral signal, which was repeatedly combined with an aversive outcome. This experiment allows us to study the memory fixation of associations of negative conditional signals with unconditional signals (Vaidyanathan, Patrick and Bernat, 2009).

3.3.4.4 Electrodermal activity (EDA)

As a method that would allow us to study the activation of the autonomic nervous system, the study of electrodermal activity is often used. The fact is that the skin can demonstrate the general arousal of the body – the autonomic nervous system regulates this mechanism through the stimulation of sweat glands. Because of this, the skin's electrical conductivity will change, and we can track the

changes during a presentation to patients stimuli related to a traumatic event. In modern practice, the methodology of measuring skin conductivity has also played a significant role in accumulating information bases in research related to PTSD. In addition, the data collected in this way are used in the context of other clinical manifestations, complementing the picture of the subject's condition, along with self-report reports and other physiological indicators (Janka *et al.*, 2015).

4. Biomarkers

4.2 Neurostructural biomarkers

As mentioned earlier, the various studies listed above can help us see the changes in brain structure caused by PTSD. Indeed, even at the initial stage of the development of this disease, we can track changes, thereby unambiguously confirming the presence of physical changes. At this stage, it is worth considering structural changes in more detail, which, among other things, will help us understand precisely how the PTSD mechanism works.

4.2.1 Hippocampus

Striking changes can be observed in the hippocampus, which performs an essential function in forming new declarative memories and consolidating short-term memories to long-term memory. It is also responsible for spatial representation and spatial memory, allowing people to construct mental maps of their environment and perform spatial tasks, such as navigation to hidden targets or finding shortcuts (Smith *et al.*, 2015). The hippocampus itself consists of two main parts – the dentate gyrus and the hippocampus itself. Here, episodic and contextual memory is declared, and the hypothalamic-pituitary-adrenocortical axis is regulated (Griebe *et al.*, 2015). This "memory centre" is also the structure with many glucocorticoid receptors, which makes the hippocampus an exciting area for studying the effects of PTSD on cognitive function (Shoshan and Akirav, 2017).

High levels of glucocorticoids produced during stress lead neurons located in the hippocampus to atrophy (Carrion, Weems and Reiss, 2007). Also, high levels of glucocorticoids affect dendrites, which lose their length and decrease in number (Conrad *et al.*, 2007). All these processes could lead to a decrease in the volume of the hippocampus. All these data are confirmed by a study that revealed the "neurotoxicity hypothesis". This hypothesis states that prolonged exposure to glucocorticoids may weaken neuronal defence's ability in stroke, leading to neuronal atrophy and/or inhibition of neurogenesis.

Reduced hippocampal volume in PTSD is a classic hallmark of the condition. Numerous studies conducted in the world prove this fact. For example, researchers tested brain volumes in twins in which one subject was affected by PTSD, and the other did not develop PTSD, and a smaller hippocampal volume was recorded (Gilbertson *et al.*, 2002). The hippocampal reduction has also been reported in Vietnam War veterans – and Bremner's study was one of the most fundamental in PTSD research (Bremner *et al.*, 1995). Data on hippocampal reduction studies are also found in modern other studies (Keding and Herringa, 2015; Sussman *et al.*, 2016).

Many PTSD symptoms were found in people with reduced connectivity between the hippocampus and the ventromedial prefrontal cortex. The study conducted with participants with childhood maltreatment has shown reduced the right hippocampal volume (Morey *et al.*, 2016). Many PTSD symptoms were seen in soldiers who participated in the research that show greater hippocampus responsiveness to stress-related content (Admon *et al.*, 2009). A follow-up study of the subjects indicated that the white matter connecting the vmPFC and hippocampus was reduced (fig. 1) after severe stress (Admon *et al.*, 2013). It is noteworthy that the connection between the mPFC and the hippocampus plays an essential role in the process of fear extinction, which is disrupted in PTSD (Lin *et al.*, 2016). If this connection is broken, the person cannot extinguish the fear, which means that every time they encounter certain circumstances that remind them of the trauma, causing reexpiriencing the traumatic event (Zhu *et al.*, 2018). Neurovisual study with MRI scan has shown a decrease in the hippocampus volume in people with PTSD compared to the control group (Bae *et al.*, 2020).

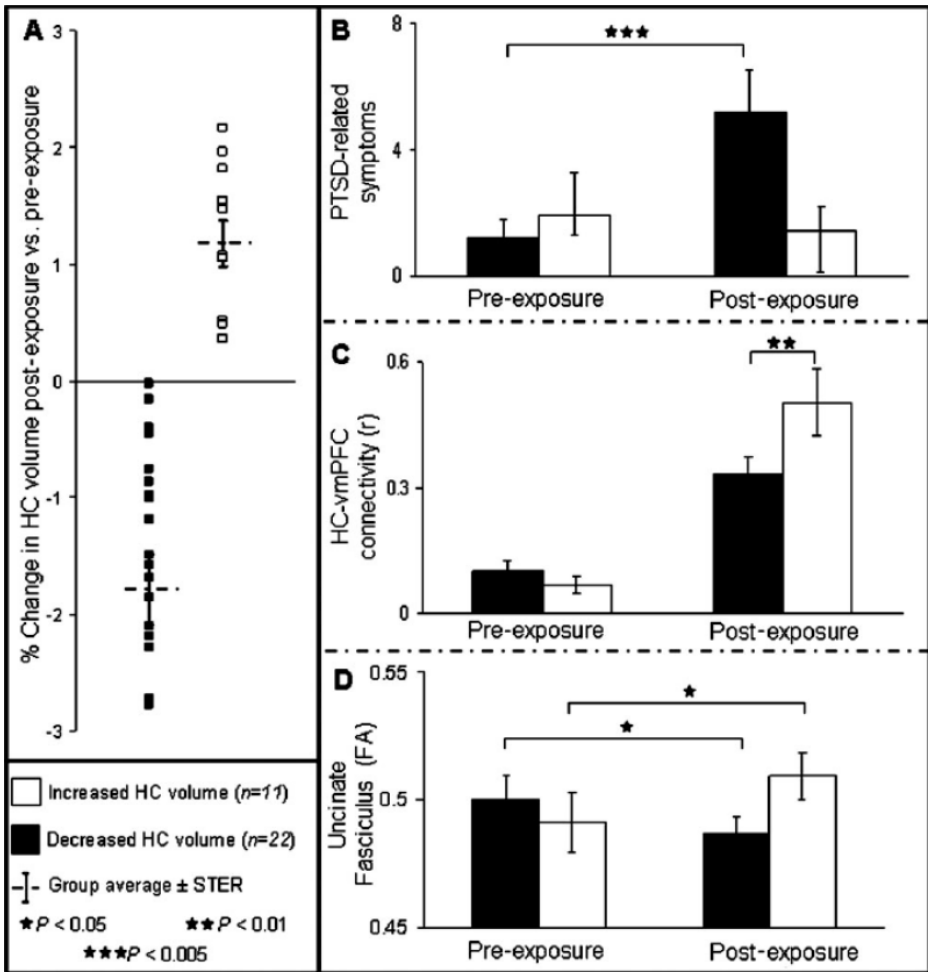

Figure 1 - Effects of stress exposure on hippocampal (HC) volume. (A) The plot shows the change in HC volume of each soldier postexposure vs. pre-exposure to stress. Soldiers were divided into two groups based on the direction of change (increased HC volume, white squares and decreased HC volume, black squares). Group averages are shown by gray cross. The individual value of HC volume change was normalized to percent change by dividing it by the individual initial HC volume, thus avoiding any effects of differences in initial HC volume. (B) Bar graphs representing the groups' average severity of PTSD-related symptom at each time point. Note that the increase in symptom severity postexposure to stress was evident only for the soldiers with decreased HC volume (P <0.005). (C) Bar graphs representing the groups' average strength of HC-vmPFC functional connectivity at each time point. Notethe overall increase in HC-vmPFC functional connectivity post-exposure to stress, which was more prominent for the soldierswith increased HC volume (P < 0.01). (D) Bar graphs repre-senting the groups' average fractional anisotropy (FA) value of the uncinate fasciculus (UF) (i.e., structuralintegrity) at each time point. Note that soldiers with increased HC volume had increased UF integrity postexposure relative topre-exposure (P < 0.05), whereas soldiers with decreased HC volume had decreased UF integrity postexposure relative topre-exposure (P < 0.05). (HC, hippocampus; UF, uncinate fasciculus; FA, fractional anisotropy; N = 33; error bars ± SEM).
(Admon *et al.*, 2013)

4.2.2 Amygdala

When looking at structural changes in the brain, we cannot just focus on the hippocampus because changes are also observed in other parts of the brain. The amygdala, an area of the brain that is part of the limbic connected with the prefrontal cortex and the hippocampus, also changes. The amygdala processes fear memory, participates in decision-making and forms emotional responses such as anxiety, aggression, or defensive behaviour (Cheng *et al.*, 2006). This part of the brain is responsible for the fight-or-flight response. In this part of the brain, the object of fear and its features are encoded, and associations are formed. In the amygdala, fear takes its form, but here it is

also "extinguished", as the action of stress hormones in the amygdala reduces anxiety (Aubry, Serrano and Burghardt, 2016).

As a result of PTSD, amygdala hyperactivity was found in soldiers before participating in frontline military operations (Breslau *et al.*, 1998). After the deployment, some of these people showed symptoms of PTSD; also, hyperexcitation of the amygdala has been documented after the military mission (Admon *et al.*, 2009). Studies conducted on police officers have also confirmed that such a reaction is a prerequisite for developing PTSD in the future (Pole *et al.*, 2009). Orphans who were traumatized in childhood had an increased volume of the amygdala in comparison with the control group (Maheu *et al.*, 2010). The exact change was recorded in children whose mothers showed maltreatment to the child (Pechtel *et al.*, 2014). An increase in the amygdala was also observed in patients with a pre-existing diagnosis of PTSD (Kuo, Kaloupek and Woodward, 2012). Other studies have shown increased functional connectivity between the amygdala and hippocampus (Baeuchl *et al.*, 2015).

4.2.3 Prefrontal cortex (PFC)

Structural changes affect, in addition to the amygdala and hypothalamus, also the prefrontal cortex of the brain. This part of the brain is located at the forefront of the cerebral cortex. It performs several functions, such as inhibition of stress reaction, speed of information processing, increased attention, planning, forecasting, and reasoning (Clausen *et al.*, 2017). Meta-analysis has shown a decrease in the strength of the prefrontal cortex in people diagnosed with PTSD (in meta-analysis Patel *et al.*, 2012). Since the prefrontal cortex is directly involved in emotion regulation and executive functions, it is reasonable to assume that changes in the volume area will affect these functions.

A morphological study found a correlation between PTSD and a reduction in the volume of the prefrontal cortex, especially in the anterior cingulate cortex (Morey *et al.*, 2016). A neuroimaging study has shown a reduced activation level in the medial prefrontal cortex and lateral prefrontal cortex, which confirmed the hypothesis of a violation of regulatory processes that contribute to the impairment of fear regulation and extinction (Clausen *et al.*, 2017).

As mentioned above, MRI is actively used in PTSD research. So, during fMRI of the brain of a patient with PTSD who actively recalled traumatic events from the past, research has found increased connectivity in the amygdala between the medial and dorsolateral prefrontal cortex (Chen *et al.*, 2018). Increased neural activation in brain regions could impair the modulation and regulation of emotions. High activity levels were recorded in the medial prefrontal cortex (Peres *et*

al., 2011) and low activity levels in the rostral anterior cingulate cortex in a study with positron emission tomography (Bremner *et al.*, 1999).

People who suffer from PTSD have dissociative symptoms. Thus, the study showed reduced activation of the ventral prefrontal cortex, which may be associated with hyper-inhibition of limbic areas in response to extreme arousal levels (St Jacques *et al.*, 2011). Young people in the age group from 10 to 16 years who were diagnosed with PTSD, when tested with the recognition of different human emotions in images, showed greater activation in the amygdala, hippocampus, the medial and ventrolateral prefrontal cortex. However, the activation level in the dorsolateral prefrontal cortex was lower than in the control group (Garrett *et al.*, 2012).

The anterior cingulate cortex (ACC) is part of the cingulate cortex. The ACC is responsible for cognitive control, autonomic monitoring and regulation, executive function, and emotional information processing (in review Stevens *et al.*, 2011). The rostral ACC is involved in the implicit regulation of emotions and is actively involved in interaction with the limbic system. At the same time, the caudal ACC is already involved in explicit cognitive regulation and regulation of human attention (in review Stevens *et al.*, 2011). This part of the brain is responsible for expressing one's own and evaluating other people's emotions, regulating attention.

So, to establish the fact of changes in the ACC volume, pairs of twins were studied. All of the twins participated in military operations, but those twins in the pair who did not develop PTSD showed higher ACC volume than those twins who were diagnosed with PTSD (Kasai *et al.*, 2008). Another twins study showed increased activity in the dorsal anterior cingulate cortex in combat twins with PTSD, then the other twins without PTSD (Shin *et al.*, 2011).

4.3 Cortisol

In addition to structural changes, a patient's body with PTSD may also be subject to hormonal changes. The most remarkable representative of this change is a change in cortisol levels.

Cortisol is a glucocorticoid hormone that is the primary regulator of carbohydrate metabolism in the body (Christiansen *et al.*, 2007). Cortisol is produced by the adrenal cortex and is actively involved during stress reactions. At the same time, cortisol also has protective functions for the body – it slows down the inflammatory process in the body (Caroprese *et al.*, 2010). Under stress, the immune system is suppressed, probably to avoid inflammatory processes occurring when the immune system is highly reactive (Miller, Cohen and Ritchey, 2002).

Cortisol optimizes the body's resources to deal with a stressor. However, despite all the benefits of this mechanism for human life, excessive stress is disastrous. Prolonged stress causes impairment of cortisol regulation in the body (Quartana *et al.*, 2010). This factor causes an increase in the allostatic load mentioned earlier, and an imbalance in regulatory systems can lead to changes of various kinds in the physiology of the body.

The most obvious example of this effect is the HPA axis, which response to increased cortisol levels by feedback under normal conditions. It reduces the secretion of corticotropin-releasing hormone and adrenocorticotropic hormone. Nevertheless, as soon as cortisol becomes abundant, the HPA axis can become passivated, thereby disrupting the normal functioning of the negative response during stress (Suliman *et al.*, 2007). Further, if the HPA axis is not restored to its normal state, the patient may develop abnormal cortisol levels (Fragkaki, Thomaes and Sijbrandij, 2016). The cortisol level tends to be lower in the case of PTSD (Dekel *et al.*, 2017). However, people with PTSD have been found to have increased glucocorticoid receptor sensitivity, which may be due to an increased number of glucocorticoid receptors (Lehrner *et al.*, 2014).

Several studies prove this fact. In some cases, a link was found between low cortisol output and increased sensitivity to negative glucocorticoid feedback in some groups with PTSD (Yehuda, Bierer, *et al.*, 2009). Conversely, some studies found no differences in cortisol or glucocorticoid feedback levels between subjects with and without PTSD (Bader *et al.*, 2014). Also, a systematic review suggests that victims of violence of various kinds do not show differences in cortisol levels between victims with established PTSD and the control group without PTSD (in review Morris, Compas and Garber, 2012).

Glucocorticoids can affect memory, particularly the hippocampus and working memory mediated by the frontal lobes (Bremner *et al.*, 1995). In addition, during a stressful situation, glucocorticoids activate PFC and the amygdala (Geuze *et al.*, 2012) by increasing the level of catecholamine release (in review Hauer *et al.*, 2014). Here one can also add that glucocorticoids inhibit the proliferation of neurons and contribute to dendritic atrophy (Liston and Gan, 2011).

Cortisol provides negative feedback in the HPA axis and has anxiolytic, sedative, anaesthetic, antinociceptive, neuroprotective and regenerative effects, and is also necessary for myelination (Nugent *et al.*, 2015). So, in studies, a dose of hydrocortisone administered to a patient helped reduce the subsequent development of PTSD after a traumatic event (Zohar *et al.*, 2011).

Disorders caused by low cortisol levels affect a large number of body functions. In particular, they relate to the inhibition of stress response, working and long-term memory, and metabolism. Thus, it

makes it an attractive biomarker for research not only on PTSD but also on other diseases related to stress and metabolism.

4.4 Inflammatory biomarkers

Changes in brain structures may also be associated with inflammatory processes caused by hormone regulation dysfunction. Therefore, the researchers paid attention to immune factors.

In patients diagnosed with PTSD, there was a decrease in the activity of the HPA axis and the parasympathetic nervous system. In contrast, the sympathetic nervous system activity increased (Smith *et al.*, 2021), which can lead to changes in the immune system and regulation of cytokine levels (Bell *et al.*, 2017). PTSD has also been linked to the incidence of autoimmune diseases, where PTSD can increase inflammation or accelerate the development of an inflammatory response (Bookwalter *et al.*, 2020).

The primary markers that indicate inflammation formed in the body are cytokines and interferons. At their core, they are vital molecules. They are a signal of the appearance of immune activation, which, as a consequence, can affect the brain. Such changes can be toxic for neurons, and the toxic effect itself is on the brain in different ways.

Glucocorticoids inhibit lymphocyte proliferation (Blotta, DeKruyff and Umetsu, 1997) and reduce the release of proinflammatory cytokines (Yehuda, Cai, *et al.*, 2009). At the same time, the activation of the HPA axis and the secretion of glucocorticoids prevents the development of an inflammatory reaction (Auphan *et al.*, 1995).

In PTSD, the effect of glucocorticoids decreases, which means that the chances of developing inflammatory processes increase. The study has also found that some potentially counter-regulatory changes in the immune system's sensitivity to the cortisol regulatory signal were observed in PTSD (Rhein *et al.*, 2021).

Inflammatory activity is also involved in developing PTSD – this statement has been confirmed (Raison *et al.*, 2013). As studies have shown, neuroinflammation occurs because peripheral proinflammatory cytokines can cross the blood-brain barrier and cause neuroinflammation (Banks *et al.*, 2002). Studies have also shown that patients with PTSD have significantly higher levels of proinflammatory cytokines in their blood (Bruenig *et al.*, 2018).

IL-6 is one of the critical proinflammatory cytokines and an essential mediator of the acute phase response in body tissue damage (Nishimoto *et al.*, 1989). In PTSD, elevated levels of IL-6 were found (Rohleder *et al.*, 2004), while another study found a decreased level of IL-6 in PTSD (M.

Plantinga *et al.*, 2013). A study has found higher levels of IL-1β in patients with PTSD compared to the control group (Gola *et al.*, 2013). However, the other study has found no significant difference in changes in IL-1β level (von Känel *et al.*, 2007).

In studies conducted on victims who developed PTSD after the earthquake and on war refugees, increased cytokine IL-8 were observed compared with the control groups (Song *et al.*, 2007).

Most data on IL-2, a cytokine central to T cell development, and its receptor (IL-2R) showed lower levels in patients with PTSD than controls (Song *et al.*, 2007).

A twin study of C-reactive protein produced by the liver in response to inflammatory signals found a significant increase in patients with PTSD, regardless of sociodemographic factors and lifestyle (L. Plantinga *et al.*, 2013).

In another animal study, the immune "priming" before trauma alters the stress response by using the predator stress model in mice. In addition, a violation of pro-/anti - inflammatory balance was found in mice (Deslauriers *et al.*, 2017).

4.5 Genetic and epigenetic biomarkers

The researchers also studied genes that may be associated with neurodegenerative changes in the brain, and they are also responsible for the sensitivity of cells to hormones. One of the features of studying genes as biomarkers are that they do not change over a lifetime.

The main areas of gene research are the noradrenergic, dopaminergic, and serotonergic systems and neurotrophins. Because these areas are related to memory and stress regulation, breaking these systems at an early age may be the "First Hit" in the "Two-Hit" hypothesis.

Bishop et al. have described FKBP5 as the protein encoded by the gene of the same name that is involved in immunoregulation and protein folding and trafficking and in hormonal and glucocorticoid signalling under stress (Bishop *et al.*, 2018).

In environmental studies of candidate genes, people exposed to childhood abuse had FKBP5 gene polymorphisms associated with high induction of the FKBP5 protein (Binder *et al.*, 2008). In addition, the FKBP5 protein increases the concentration of glucocorticoid receptors, leading to increased sensitivity of cells to glucocorticoids, observed in PTSD (Klengel *et al.*, 2013).

Studies of the regulation of the FKBP5 gene, a few hours after injury, increased level of the FKBP5 gene has predicted the development of PTSD (Binder *et al.*, 2008). However, the opposite results were carried out on victims of the September 11 terrorist attack on the World Trade Center. In

contrast to the control group, people who subsequently developed PTSD had a lower concentration of the FKBP5 gene (Yehuda, Cai, *et al.*, 2009).

In the largest GWAS study involving a sample of 20,730 people, the researchers found no significant single-nucleotide polymorphisms associated with PTSD (Duncan *et al.*, 2018).

GWAS studies on women revealed a link between PTSD and a single-nucleotide polymorphism in the SLC18A2 gene, associated with monoamine transport (Solovieff *et al.*, 2014). Another study showed new variants of the NR1F1 gene, which is associated with regulating sleep and cytokines (Logue *et al.*, 2013).

A special place is occupied by studies of epigenetic modifications that can activate or deactivate genes under the influence of the environment, thereby changing their function. It is also worth considering epigenetic changes as a potential "hit" in a “Two-Hit” hypothesis, leading to PTSD.

DNA methylation is one of the main mechanisms of epigenetic regulation or genetic functions mediated through mechanisms independent of DNA sequences. Therefore, consideration of epigenetics is interesting, including the transmission of a predisposition to PTSD from parents to offspring.

Methylation of genes involved in immune regulation showed an increased concentration of some cytokines associated with immune system reactivity IL4, IL2, and TNFalpha among people with PTSD (Uddin *et al.*, 2010).

One of the genes under consideration is SLC6A4, which encodes a serotonin transporter involved in regulating serotonin availability in the brain (Tao-Cheng and Zhou, 1999) and regulating emotional aspects of behaviour (Jonassen *et al.*, 2012). Studies of the methylation SLC6A4 gene may clarify disorders associated with stressor assessment in patients with PTSD (Alexander *et al.*, 2014). So, studies have shown its effect on the activity of the amygdala when presented with fear stimuli (Hariri *et al.*, 2005).

A study conducted by Pinna et al., using the social isolation model in rats, showed an alteration in corticolimbic GABAergic neurotransmission (GABA receptor). Also, it downregulated corticolimbic allopregnanolone concentrations and altered the neurocircuitry of fear (Pinna and Rasmusson, 2014).

Georgopoulos et al. base their work on the principles of the “Two-Hit” hypothesis described earlier. Thie work presents a model consisting of neuroimmune stress as a pre-existing condition, "First Hit". It means that when confronted with a traumatic event, an increased glutamatergic response

"Second Hit" is inherent. Together with an increased neuroimmune load, it leads to an increased level of ICAM-5, which regulates glutamatergic neurotransmission and local immunosuppression. Thus, ICAM-5 "closes" the circuit by reactivating the glutamatergic system (Georgopoulos *et al.*, 2018).

In support of the significance of the “Two-Hit” hypothesis in the development of PTSD, Mancini et al. demonstrated an animal model experiment in which rats are exposed to social defeat as an analogy to the "First Hit" adverse experiences in humans at an early age, which in combination with single prolonged stress as a late stressful event "Second Hit" which leads to the development of stress-related psychopathologies. The "Second Hit" lead to an increased concentration of BDNF in the hippocampus and a reduced corticosterone level (Mancini *et al.*, 2021).

4.6 Psychophysiological biomarkers

Psychophysiology research primarily focuses on the sensitivity of patients with PTSD to stressful stimuli. Patients with PTSD often experience hyperexcitation, increased aggression, and problems with concentration (American Psychiatric Association, 2013). Electrophysiological methods discussed earlier help to investigate biomarkers associated with the autonomic nervous system.

A study on people with PTSD measured their heart rate, facial muscle myography, and skin conductance while listening to audio recordings associated with traumatic memories. Patients with PTSD showed increased responsiveness of the sympathetic nervous system to the effects of a stressor compared with victims without PTSD (Orr *et al.*, 1993). In addition, a study with veterans and military service members has shown decreased high-frequency heart rate variability and reduced parasympathetic modulation with PTSD (Powers *et al.*, 2021).

Delayed extinction by lower skin conductance response has been found in patients with PTSD by measuring their electrodermal activity during a threat of shock (Blechert *et al.*, 2007).

Patients with PTSD may experience symptoms of chronic fatigue. In a study of 18 military personnel, half of whom had PTSD, the technique of electromyography was used. Results showed a significant main effect on electrical activity and neural conduction variables in the PTSD group (Sarabzadeh, Soleimanifar and Helalizadeh, 2020).

5. Discussion and Conclusion

5.2 Discussion

Intense stressors can disrupt the balance of the body's systems, leading to the development of PTSD. Various biomarker studies shed light on the aetiology of PTSD.

In the case of PTSD, the aetiology of development is not clear at the moment, despite a large number of studies conducted. This may tell us about the large variability in the development of PTSD and individual predispositions to the development of trauma, which has yet to fall into the group of clinical biomarkers that will help avoid the development of post-traumatic stress disorder. Even though PTSD is the one disease, it has a different course in patients and symptoms that overlap with other affective diseases. At the same time, it also has its predispositions to development, which in combination can lead to disease ("Two-hit" hypothesis).

Thanks to previous studies, we understand what functions are responsible for different brain structures. So from the very beginning of the study, we observe external manifestations of violations of the body's normal functioning in the form of symptoms. Next, scientists consider organs, the structure of different parts in the organ that can potentially be associated with this type of disorder. So, scientists study the tissue, cells, and genes; they are going into the root of the problem by studying biomarkers, scientists find patterns of disease manifestation. On the other hand, we should not forget the body as a balanced system, supporting each other in a normal state.

Allostatic load leads to an imbalance of the body's regulatory systems. The imbalance leads to a mass of the changes that are biomarkers of PTSD, and because of which we can see various symptoms of PTSD. For example, patients with PTSD have symptoms of dissociation, avoidance of potential triggers, memory impairment and depersonalization, and involuntary memories of the traumatic event and flashbacks.

These symptoms lead us to research structures connected with this type of functional impairment. Depersonalization and memory problems are symptoms associated with the amygdala, hippocampus and insula. Therefore, the researchers conducted neuroimaging studies searching for biomarkers that showed increased hippocampal volume (Gosnell *et al.*, 2020), amygdala activity changes (Gosnell *et al.*, 2020) and increased insula functional connectivity in PTSD (Harricharan *et al.*, 2020), indicating insufficient memory consolidation about the time and place of the traumatic event. Symptoms of depersonalization and low emotional level may be associated with the prefrontal cortex and anterior cingulate cortex disorders. In addition, the low self-esteem and self-

blame that occur in PTSD may be associated with a detected violation of the monoamine transporter, in particular the serotonergic system of the brain (Forbes *et al.*, 2014).

Due to an impairment of the amygdala activity, a person who is subsequently confronted with a trigger (conditioned stimulus) associated with trauma involuntarily mentally "returns" to experiencing trauma, reexperiencing similar stress reaction as when they experienced the situation initially (Cheng *et al.*, 2006). Furthermore, the subject's aggressivity may increase, resulting from increased amygdala activity in mice (Qi *et al.*, 2018). The above data are supported by a psychophysiological study of the autonomic nervous system's increased reactivity in PTSD (Sarabzadeh, Soleimanifar and Helalizadeh, 2020).

One of the manifestations of an imbalance of systems due to allostatic load is a reduced cortisol level, while an increased concentration of glucocorticoid receptors is detected in the body (Shoshan and Akirav, 2017). Conversely, disruptions in cortisol-mediated feedback can lead to high levels of catecholamines and increased arousal of systems related to memory and emotion regulation. From these facts, we can conclude that the imbalance of one system is closely related to the imbalance of another system, creating a vicious circle.

Structural brain degenerations may have an autoimmune origin, not just hormonal or physiological, evidenced by an increased level of proinflammatory cytokines in PTSD. This fact is proved by increasingly emerging studies that study the predisposition to PTSD using the "Two-Hit" hypothesis. As a ”First Hit", scientists investigate the impact of early stress through the formation of the immune system, the effects of stress on the nervous system. The "Second Hit" is a severe injury that takes the body out of allostasis.

While working on the review, some difficulties were interpreting the results and their generalization to a larger population. For example, most studies of cortisol levels are conducted on men, in addition to the fact that it is essential to create a design that considers changes in hormone levels throughout the day and allow experiments conducted on women, which are inherently more complicated due to the significant variation in the level of the hormone during menstrual cycles, pregnancy. Furthermore, when working with people from different backgrounds, it is essential to consider their cultural characteristics and mentality. For example, it is not acceptable to spit in some cultures, which means that an experiment based on saliva analysis can be complicated.

The development of independent research laboratories that can be deployed directly at crash sites or other places where large-scale traumatizing events occur will help monitor biomarkers and consider the mechanisms in the body immediately after injury.

When studying PTSD, there is individual variability in the aetiology of trauma development. The good news is the development of artificial intelligence and computerization that allows one to process large databases (Schultebraucks *et al.*, 2021). At the moment, there are already neuroimaging studies using artificial intelligence, which helps to find structural changes in PTSD images (Nicholson *et al.*, 2019). However, even though it becomes easy to process meta-analyses, this process is complicated by different standards for setting the design of the experiment and the difference in diagnostic criteria.

It is difficult to interpret the results obtained in animal models since they overlap with other affective disorders (Carmassi *et al.*, 2020). Moreover, they only partially overlap the PTSD phenotype, not responding to multi-level disorders accompanied by the tension of all organs and systems that would reflect disorders in real stress trauma.

To increase the experiment's validity, the design of the experiment should take into account individual characteristics of the person, such as gender, hereditary diseases, substance use, childhood injuries, social loneliness, and the economic situation, since these factors affect the result and interpretation.

It is worth considering PTSD as a complex disorder affecting a large number of regulatory systems. Further research using GWAS will help us discover previously unknown biomarkers of PTSD and bring us closer to understanding the aetiology of PTSD. Studies of biomarkers and methods of their analysis will make it possible to introduce rapid tests to identify people at risk, which will avoid work injuries and select suitable personnel for work in conditions of increased stress. In addition, the introduction of PTSD biomarkers into clinical practice may lead to the development of a personalized treatment system.

5.3 Conclusion

PTSD can affect the life of every person on this planet at absolutely any time of their life. Only one traumatic event that has happened to us can cause this severe mental illness. It should be understood that a patient suffering from PTSD is not capable of everyday functioning in a social environment since his life is entirely overshadowed by a negative experience, outbursts of anger, problems with sleep, and difficulties in concentration. Furthermore, it is essential to study both PTSD in general and biomarkers that can help detect PTSD or its treatment.

However, to create a methodology for the treatment and prevention of PTSD, it is crucial to understand the aetiology of this phenomenon. Despite the apparent simplicity, it is not so easy to implement this because, at the moment, the aetiology is not fully clear to the scientific community.

At this point, information about biomarkers and their research comes to the rescue. Moving in this direction allows scientists to gradually find clues to the secrets of many of the processes behind the emergence and development of PTSD.

This thesis describes methods of preclinical research in animals and clinical methods of research in humans, which allow the scientific community to track and identify the mechanism of action of biomarkers in PTSD. Further, a review of biomarkers was conducted that showed structural disorders of the hippocampus, amygdala, prefrontal cortex and anterior cingulate gyrus and changes in the functioning of the autonomic nervous system. We also reviewed studies showing that cortisol levels are lowered and that it affects brain structures. In studying the above information, it was concluded that an inflammatory reaction of the body might be behind neurodegenerative processes, which, along with a genetic predisposition, may indicate the development of PTSD.

Further development of biomarker research methods will allow us to more accurately identify essential biomarkers for the development of treatment and prevention of PTSD. New technologies and artificial intelligence for measuring patterns of changes in brain structures during neuroimaging and simplifying the processing of large databases will allow researchers to consider the aetiology of PTSD more broadly by processing a large number of biomarkers in the aggregate. Research using the GWAS method can detect new genetic biomarkers.

However, even though the above-mentioned modern methods are widely used today. There are still many "dark spots" in the environment of biomarkers and the general definition of the aetiology of PTSD, and we would like to hope that a way out of this challenging situation will still be found. Thus, thousands of people currently suffering from PTSD will receive decent help.

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