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REVIEW

Could a blood test for PTSD and depression be on the horizon?

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ABSTRACT

Introduction: Depression and posttraumatic stress disorder (PTSD) are two complex and debilitating psychiatric disorders that result in poor life and destructive behaviors against self and others. Currently, diagnosis is based on subjective rather than objective determinations leading to misdiagnose and ineffective treatments. Advances in novel neurobiological methods have allowed assessment of promising biomarkers to diagnose depression and PTSD, which offers a new means of appropriately treating patients.

Areas covered: Biomarkers discovery in blood represents a fundamental tool to predict, diagnose, and monitor treatment efficacy in depression and PTSD. The potential role of altered HPA axis, epigenetics, NPY, BDNF, neurosteroid biosynthesis, the endocannabinoid system, and their function as biomarkers for mood disorders is discussed. Insofar, we propose the identification of a *biomarker axis* to univocally identify and discriminate disorders with large comorbidity and symptoms overlap, so as to provide a base of support for development of targeted treatments. We also weigh in on the feasibility of a future blood test for early diagnosis.

Expert commentary: Potential biomarkers have already been assessed in patients' blood and need to be further validated through multisite large clinical trial stratification. Another challenge is to assess the relation among several interdependent biomarkers to form an axis that identifies a specific disorder and secures the best-individualized treatment. The future of blood-based tests for PTSD and depression is not only on the horizon but, possibly, already around the corner.

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1. Introduction

Coping with stress and negative life experience is an essential survival function not only in humans, but also in all living organisms. Environmental factors affect behavioral regulation and the ability to adapt to changed environmental conditions or the lack thereof, could play a key role in the onset of mood disorders. While adversities are very frequent during the lifetime of an individual, ranging from 50% to 84% in the general population [1,2], the majority of people are resilient to adverse life events [3]. Nonetheless, a large portion of them (~10%) fails to develop resilience, and develops, instead, mood disorders, such as major depressive disorder (MDD) and/or posttraumatic stress disorder (PTSD) [4].

MDD and PTSD have been dramatically increasing in the past decade [5]. Depression is a profound multifaceted neurobiological disorder of mood and emotions, which is often the result of different psychological stressors, has a prevalence of 8–12% [6], and is the cause of impairment in different neuropsychological functions, like attention, learning, and memory [7]. The DSM-5 describes a multitude of symptoms for MDD, such as sadness, anhedonia, disturbed concentration, significant changes in body weight (loss or gain) that highlight the complexity of this neuropsychopathology [8]. Likewise, PTSD is a stress-induced psychiatric disorder that emerges in individuals after the exposure to a trauma and is characterized by an altered ability to cope with stress. The core symptoms of this psychiatric disorder are reexperiencing symptoms, nightmares about the trauma, changes in arousal and reactivity, avoidance of trauma-related reminders and alterations of mood [8]. PTSD and MDD share numerous overlapping symptoms and comorbidity and often MDD symptoms in PTSD patients are a progression of the disorder [9]. Both PTSD and MDD are characterized by high incidence of suicidality with a prevalence of 9.5% of patients with MDD that attempted suicide over an 18-month period [10]. Predictors for suicide in MDD patients are the male gender, family history of psychiatric disorders, more serious depressive symptoms and comorbidity with other disorders, such as anxiety spectrum disorders and substance use disorder [11]. The core and the overlapping symptoms of MDD and PTSD are presented in Figure 1.

Moreover, both these neuropathologies show a gender-related dimorphism with females more affected than males [12,13]. In fact, in both PTSD and MDD, the prevalence in women is more than double that observed in males [14] pointing to a role of sexual hormones in the development and maintenance of the disorder. While, imbalance in sexual hormones synthesis could play an important role, they cannot explain the basic psychobiological mechanisms. Recently, it has become clearer that the convergence of multiple factors, such as

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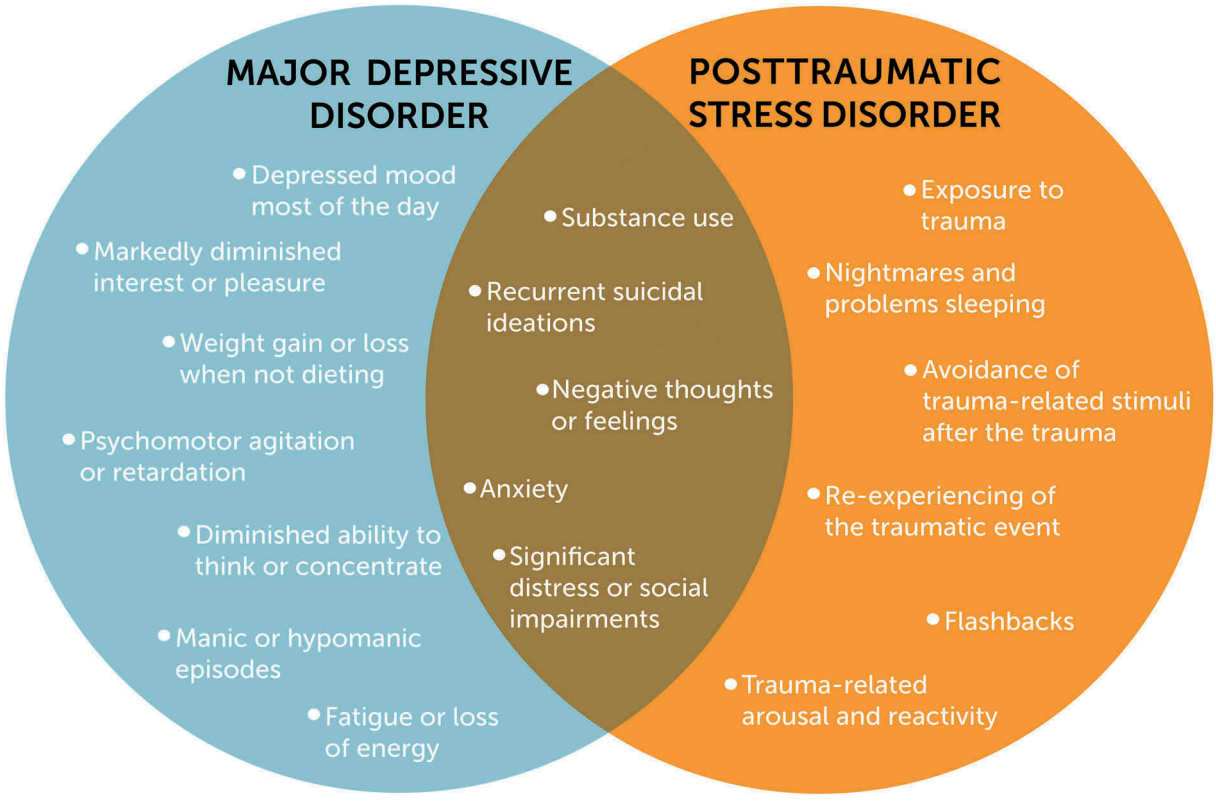

Figure 1. Core symptoms of MDD and PTSD described by DSM-5.

The DSM-5 describes the criteria to diagnose psychiatric disorders such as MDD and PTSD. The light blue circle lists the core symptoms of MDD, while, the orange circle lists the core symptoms of PTSD. The overlapping area represents the shared symptoms of these two disorders.

biological aspects and sociopsychological environmental conditions, play a role in MDD and PTSD [15–18]. Likewise, a multiplicity of factors, including genetic vulnerability, immunological alterations, epigenetic mechanisms, neurohormones, neurotransmitter systems, neuropeptides and endocannabinoids and their receptors, seem to play a role in both the manifestation and the maintenance of mood disorders.

Despite advances in the neurobiological and pathophysiological aspects, the current diagnosis for MDD and PTSD is based on patients' self-reported interviews and on the clinician's observation. These methods to diagnose psychiatric disorders are quite subjective and far from optimal because of the consistent heterogeneity not only of the symptoms, but also of the disorder etiology [19]. Furthermore, patients with mood disorders frequently are not able to adequately characterize and describe the symptoms of the pathology [20] and the scoring systems are often discordant [21]. This scenario underscores the need to develop objective biological diagnostic tools based on neurobiological deficits to obtain early diagnosis, monitor individuals at risk, instruct individualized treatments, follow-up on treatment success and possibly prevent future relapse [22,23]. We anticipate that this will bring a disruption in the diagnosis of psychiatric disorders as we know of, based on symptoms characterized by the DSM-5 to regroup disorders based on their neurobiological characteristics. The progress in the diagnosis and treatment of psychiatric disorders based on biomarker assessment, rather than by symptoms classification, is long needed. Contrary to other neuroscience fields, such as the neurodegenerative disorders, progress in the assessment of valid biomarkers in psychiatry has been rather slow [24].

In this review, we will summarize several promising biomarkers in PTSD and MDD and weigh in on whether these offer hope in the development of a future blood test for these conditions. Tests will provide a useful tool for treatment selection and to monitor treatment outcomes; ensuring patients receive the most appropriate and efficient treatment for a better chance to promptly recover.

1.1. Mood disorders and biomarker discovery

The identification of biomarkers in clinical psychiatry may give a measurable indicator of the neurobiological individual condition independently of a DSM-5-based diagnosis, of its predisposition to develop psychiatric disorders or the current presence of a behavioral dysfunction.

Biomarkers are objective measures of physiological and pathological processes or biological responses to a therapeutic intervention [25]. Biomarkers may include a gene or a set of genes, morphological characteristics, proteins, neuropeptides, or other biomolecules that are specifically altered in a disorder or disease [25]. Molecules identified as potential biomarkers allow accurate diagnosis and prognosis, to understand the pathogenesis and the pathophysiological mechanisms of the disorder, to predict disease

progression, and to monitor the therapy progress. Biomarkers can also be useful to identify drug targets for the development of new therapeutic strategies [26].

Diagnostic tests are an urgent goal in the development of biomarkers for psychiatric disorders, because these tests will provide an objective approach to distinguish not only healthy individual from whom is affected by the disorder, but also can help classify correctly the subpopulations of patients with or without comorbidities. The use of imaging together with new molecular and genetic technological approaches can offer an important opportunity to supplement the current subjective symptom-based diagnosis [27]. However, the development of screening tests for psychiatric disorders is still remote. The only screening tests currently usable for psychiatric disorders are behavioral assessments, which are associated with an individual's higher risk in developing the disorder (e.g. family history of mental disorders; gender; substance abuse), and the investigation of discrete genetic alterations [27].

Familial or twin studies, indeed, have shown genetic predispositions for PTSD or MDD [28], which highlight the role of genetic polymorphisms in some psychiatric disorders. In particular, some polymorphisms of the dopaminergic and serotoninergic circuits have been found. A polymorphism for the dopamine D2 receptor seems to contribute to a PTSD predisposition [29], while, the serotonin transport gene is linked with more depressive symptoms and suicidal ideations [30]. The results of these early studies have not yet been replicated and their clinical implications remain premature. While these studies confirmed a possible implication of the dopaminergic and serotoninergic systems in psychiatric disorders, not even a single SNP has currently been associated with MDD or PTSD by large genomic-wide association studies. The lack of a direct link between single gene alterations and the disorders is probably due to the researcher's oversimplified models, which fail to take into account the interaction between brain, environment, and gene expression networks [31]. However, one of the principal neuronal deficits, which was firstly pointed as a primary deficit in depression, was the serotoninergic neurotransmission, although this hypothesis was never found to reach a convincing scientific consensus [32]. A fundamental evidence for the original hypothesis of serotonin's role in depression derived from the mechanism of tricyclic antidepressant (TCA) on the reuptake of serotonin. This gave rise to develop a number of antidepressants, such as the selective serotonin reuptake inhibitors (SSRIs), which, by blocking the serotonin transporter, enhance the activity of serotonin in depressed patients' brain and thereby improve their depressive symptoms [33,34]. However, SSRIs only improve symptoms in 50% of MDD and PTSD patients [35] and this disappointing result highlights that more and better biomarkers need to be discovered.

Given that PTSD and MDD are multifactorial disorders, it is important to establish a number of biomarkers and treatment targets for these disorders so that patients can be classified based on their specific biochemical deficits. Furthermore, this approach is relevant in that it may enable the stratification of patients' subpopulations, allows the design of better clinical trials and thus the testing of better treatments. Therefore, if a patient has a deficit in a specific neuroactive steroid level, an individually designed therapy will supplement that deficient neuroactive steroid, its surrogate, or an agent that stimulates its synthesis. It will not provide a serotonergic molecule that may not help and may only result in unwanted side effects.

Recently, biomarker discovery for MDD and PTSD has suggested a number of neurochemical deficits in preclinical and clinical settings, including hypothalamic–pituitary–axis (HPA) alterations in response to stress, epigenetic changes, variations of immune response or signalling, and dysregulation of the endocannabinoid and neurosteroid systems, which will be examined in further detail in the sections to follow.

2. Biomarker candidates in PTSD and MDD

2.1. Epigenetic biomarkers

In PTSD and MDD, exposure to a trauma can epigenetically impact neuronal function and affect the neurophysiological and behavioral mechanisms of stress response and adaptation [36]. The phenotypes of PTSD and MDD emerge from the complex interaction between multiple genetic and environmental factors. Epigenetic changes consist of numerous biochemical processes, including DNA methylation and hydroxymethylation, histone posttranslational modifications (PTMs), and noncoding microRNAs. All these processes are influenced by the exposure to the environment and affect the transcriptional function of genes [37]. In absence of nucleotide DNA changes, epigenetic alterations can provide a molecular mechanism for the development of various phenotypes after traumatic events. However, trauma exposure does not necessary lead to the development of PTSD or MDD. Environmental factors that lead to psychiatric disorders can be precipitated by other factors, such as genetic predisposition, timing of exposure or previous epigenetic modifications due to previous traumatic events [38,39]. To date, most epigenetic studies have examined the methylation status of cytosine residues in genomic DNA. DNA methylation, indeed, can be embedded by early life adversities or inherited through generations [40–42]. Enzymatic methylation and demethylation processes dynamically regulate the methylation of target genes, although some stress-induced epigenetic changes may become permanent [43–45].

Exposure to early life traumas is very frequent in the psychiatric patients' history [46,47]. During childhood, the programming of the neurobiological system and the most relevant epigenetic alterations take place [48]. Thus, an early adverse experience, like child abuse and neglect, dramatically enhances an individual predisposition to develop depression or other psychiatric disorders later in life. Rodent studies that have been translated to humans showed that early life maltreatment alters the epigenetic programming of the HPA. For example, the histone PTMs affecting the epigenetic status of the glucocorticoid receptor (GR), gene NR3C1 in the hippocampus of the rat varies according to the level of parental care of pups [49]. This evidence has been confirmed by human studies in which suicide victims with childhood abuse history showed changes in methylation level of NR3C1 compared to suicide victims without reported childhood abuse [50]. Moreover, the amplitude of DNA methylation changes affecting NR3C1 in adults with a psychiatric disorder diagnosis has been correlated with the repetition and the severity of the maltreatments during childhood [51].

Beside the HPA axis, the epigenetic status of other systems involved in neuropsychiatric disorders has been investigated in relation to early life stress, such as the brain-derived neurotrophic factor (BDNF) gene. The level of methylation of the BDNF gene is higher in peripheral blood cells of individuals who received low parental care [52]. Moreover, a hypermethylation of this gene has been detected in blood of depressed patients [53].

The advancement in genome-wide analysis has led the research beyond the gene-candidate approach toward a more comprehensive mapping of the neuroepigenome. The sensitivity and stability of the epigenetic alterations make them promising biomarker candidates. For example, a longitudinal study showed an association between psychiatric disorders, the amygdala–hippocampus volume ratio and the methylation of the SP6 gene, making the DNA methylation an epigenetic predictor of a specific disorder [54].

Currently, besides the status of the DNA methylation, the investigation of microRNAs (miRNAs) is becoming an increasingly focus of investigation for their functional relevance in several pathological conditions. miRNA are members of a class of noncoding RNA, transcribed from introns or exons of noncoding RNA by polymerase II [55]. Preclinical and clinical studies have shown an association between miRNA–mRNA interactions and depression or PTSD. A polymorphism of miR-30e, indeed, is positively correlated with the symptomatic onset of depression [56,57]. In the blood of male combat veterans with PTSD, eight miRNAs have been found to be differentially expressed, with four upregulated and four downregulated, compared to other healthy military personnel [58]. In the peripheral blood mononuclear cells, Bam and colleagues found higher DNA methylation in male combat veterans with PTSD and identified an altered expression of 190 miRNAs, among which 183 were downregulated [59]. Male and female combat veterans with PTSD showed decreased miR-125a and miR-181c levels [60], while mi-R3130-5p is decreased in patients with PTSD and comorbid depression of both sexes [61]. Both the increase and the decrease of miRNA function are associated with a depressive psychopathology. The overexpression of miRNA leads to an excessive silencing of several targets, while impairment of miRNA expression leads to over-translation of mRNA, affecting neuronal physiology [55]. The capability to mediate multiple targets makes miRNA a potential therapeutic strategy to recover from the chemical imbalance of patients with psychiatric disorders, but caution is necessary to avoid unexpected effects of altered miRNA expression.

Most of the epigenetic studies have failed to adequately address the timing of exposure to trauma, the temporal relationship with the epigenetic alterations and the onset of PTSD or MDD. Genome-wide studies highlighted the importance of early trauma in the epigenetic changes: patients with different traumatic histories showed distinct DNA methylation profiles and different blood gene expression. Understanding the genetic vulnerability of factors that contribute to psychiatric illness may enable the identification of individuals who fail to cope with traumatic events (depicted in Figure 2). Traumas clearly induce epigenetic changes with short- and long-term effects on neuronal function, brain plasticity, and behavioral modifications [36,62,63]. The reversibility of the epigenetic alterations makes them potential biomarker candidates for the development of new treatments for psychiatric disorders, as demonstrated by studies in rodents [64].

2.2. Chronic stress and role of the HPA axis

Aside from a genetic vulnerability, one of the major causes of depression and PTSD is the exposure to chronic and repeated

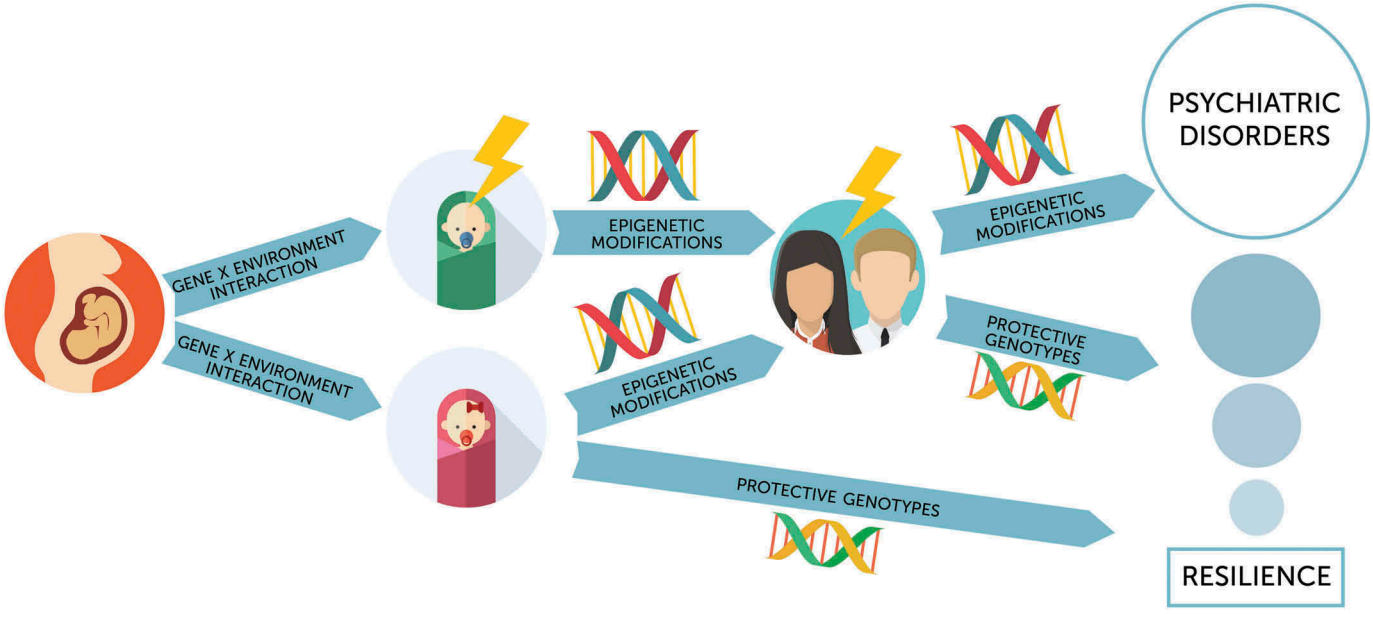

Figure 2. Genomic and epigenetic role in the development of psychiatric disorders.

The early life experiences, particularly early life adversities, may lead to epigenetic alterations. In absence of traumas and stressful conditions, there is an increased possibility of developing resilience in adult life. The exposure to early life adversities in childhood may result in a phenotype that is more susceptible to psychiatric disorders following a more recent exposure to a stressor. Nevertheless, even trauma-exposed adults who have not experienced adversities during early life are susceptible to develop PTSD and/or MDD.

stress. Prolonged stress leads to alterations of behavior and physiology of both humans and rodent models of depression and PTSD [65,66]. The stress response is mediated by the HPA axis; whose role in MDD and PTSD has been consistently demonstrated [67]. The stress response is mediated by the rapid activation of the sympathetic nervous system, which leads to the release and increase of noradrenaline and adrenaline. Blood concentrations of adrenal glucocorticoids also rise after exposure to stressors. Alterations of the corticotropin-releasing hormone (CRH) function have been reported and enhanced levels of CRH have been found in the cerebrospinal fluid (CSF) of depressed patients [68]. The increased release of CRH results in a greater secretion of adrenocorticotropic hormone (ACTH) and in increased glucocorticoid synthesis, in particular cortisol, which has an inhibitory feedback on CRH and ACTH through the GR and the mineralocorticoid receptor (MR). The affinity of these two receptors for glucocorticoids is different: MR maintains its activation for 1 h after the hormone release, while GR is progressively activated during stress. When the stress is prolonged, gene-mediated corticosteroid effects take over through the transcriptional regulation of specific sets of genes by the MRs and GRs. The functions of different proteins, indeed, are altered by the corticosteroid-mediated stress response. Of note, patients with MDD and PTSD show enhanced levels of cortisol in saliva, plasma and urine, and an increased size and activity of pituitary and adrenal glands [69]. The basal cortisol concentration has been associated with the induction of hippocampus long-term potentiation (LTP) that is implicated in memory formation [70]. On the contrary, stress, new environment, and high corticosteroid levels impair LTP and facilitate long-term depression [71]. The inability to face stressful life events leads to hypersecretion of corticosteroids, increasing the risk for psychiatric disorders in vulnerable individuals [72]. Chronic stress exposure induces an enhanced production of CRH mRNA in the paraventricular nucleus (PVN). The exact mechanism driving the increases in CRH gene expression is not completely understood, probably, it involves the repeated activation of cAMP [73]. In healthy individuals under chronic stress conditions, CRH coordinates adaption by inducing release of corticosteroids; however, this mechanism fails during depression due to an altered expression of GR levels and to a disruption of the physiological activity of the HPA axis [74]. The receptor subtype for CRH, CRHR1, seems to have important implications on anxiety and depression [75]. Papiol and colleagues showed that homozygous patients for TT in the SNP of CRHR1 gene, rs 110402, present an earlier onset of the disease and thus this SNP has been suggested as a biomarker of acquired vulnerability [76].

Several studies evaluated the pituitary negative feedback inhibition of the HPA axis by stimulating the GR by the administration of the synthetic glucocorticoid, dexamethasone (dex). The dexsuppression test was elaborated as a diagnostic test for depressed patients with comorbid melancholia, but without consistent results because the same neuroendocrine alterations in this subgroup also occur in patients with different diagnosis [77]. Patients with different mood disorders show elevated plasma cortisol concentrations after an oral administration of dex, presumably due to impaired functions of GR and MR in the pituitary [67]. By contrast, in healthy individuals, dex leads to an inhibition of the HPA axis and to decreased cortisol levels for up to 24 h [78]. The high cortisol non-suppression after dex administration has been linked with early relapse and poor clinical response in MDD [79]. Furthermore, a refined test combining dex administration and CRH stimulation (the dex/CRH test) showed that both CRH and vasopressin drive HPA activity in depressed patients [80]. Elevated cortisol response to the dex/CRH test has been observed in MDD patients. Antidepressant treatments reduce the release of cortisol after the dex/CRH test, probably because antidepressants improve corticosteroid receptor function and can regulate HPA axis activity [67]. All these findings led to the hypothesis that a restoration of corticosteroid receptor function results in a better HPA axis regulation, which precedes a clinical amelioration. In healthy individuals with high genetic load for depression, the dex/CRH test showed higher cortisol response than in individual without this genetic background [81]. This evidence highlights that changes in the HPA axis are genetic traits that increase the risk for MDD [74]. The dex/CRH test failed to show abnormalities in HPA axis function in male veterans with PTSD compared to trauma-exposed male veterans without PTSD [82]. In PTSD patients with comorbid MDD, the ACTH response to the test is attenuated compared to PTSD patients without MDD, suggesting the presence of subpopulation in PTSD with different HPA axis regulation. By comparing studies on individual with PTSD or with MDD, the dex administration leads PTSD patients to a higher cortisol suppression, while in MDD patients leads more frequently to cortisol non-suppression [82]. Altered HPA axis function in PTSD may be more related to reduced basal cortisol level and enhanced CRH activity, while in MDD, HPA axis abnormalities are probably due to reduced GR responsiveness and increased cortisol levels [74,83].

The glucocorticoid system is a potential relevant target for MDD and PTSD therapeutics. For example, GR sensitivity is regulated by FKA-binding protein 51 (FKBP5) and depressed patients exhibit decreased GR sensitivity with a substantial reduction of FKBP5 mRNA expression. The FKBP5 gene transcription is dependent on GR activation with a feedback modulation that regulates GR sensitivity. After an administration of dex, depressed patients showed a GR-mediated alteration in gene expression compared with healthy controls [84]. However, the FKBP5 gene appears to play a primary role in psychiatric pathologies and it is an important predictive biomarker. For example, FKBP5 polymorphisms associated with early traumatic events, like childhood abuse, predict adult major depression, and PTSD [85]. Of note, adversities during childhood influence the transcriptional activity and the state of HPA axis genes implicated in the response to stress. Moreover, the onset of PTSD after trauma exposure is associated with pre-traumatic biomarkers, such as higher GR number in peripheral blood monocytes or low mRNA levels of the GR-inhibitor FK506-binding protein 5 (FKBP51), which reveal the level of sensitivity to stress [86–88].

A study of Binder and colleagues showed that three polymorphisms of this gene are potential diagnostic biomarkers because significantly associated with an enhanced FKBP5 expression, which regulates the HPA axis function. Patients with these polymorphisms show less hyperactivity of the axis during depression. Moreover, rs1360780 was strongly associated with faster antidepressant response in patients, which plays a pivotal role as biomarkers to predict the treatment outcome [89].

The interaction between early traumas and polymorphisms determines the methylation state of the gene and regulates the sensitivity of FKBP5 to GR regulation [39]. Remarkably, the severity of PTSD is associated with the level of FKBP5 gene expression; low expression of this gene is linked to low plasma cortisol [90]. Several studies suggest that the low cortisol levels can regulate the FKBP5 expression through changes in the GR responsiveness [91]. By contrast, a study on PTSD patients exposed to the World Trade Center attack showed that alterations in FKBP5 may facilitate GR responsiveness, deceasing cortisol levels [90]. However, the results of this works are limited by the small subsample of patients (20 Caucasians) with current PTSD, which make it hard to distinguish between genes associated with risk, recovery, and resilience. For this reason, the findings of this early study should be replicated including a cohort of remitted PTSD patients and focusing on the function of other relevant genes, such as GR gene or the nuclear factor I/A, a transcription factor that acts in concert with GR, whose expression has been found altered in PTSD patients [90].

In another recent study, Yehuda and colleagues suggest two possible stable epigenetic biomarkers for PTSD: GR and the FKBP5 gene methylation. PTSD patients, classified as responders, showed a greater average number of methylated site at pretreatment assessment compared to nonresponders, but the methylation of the GR gene was not altered at posttreatment or follow-up. On the contrary, the methylation status of FKBP5 gene decreased in association with recovery. The GR exon 1F promoter methylation predicted the outcome of the treatment, while, the cytosine methylation of FKBP5 promoter seemed to be associated with treatment outcome [92]. It is important to highlight that the GR gene methylation does not change over time signifying that early environmental experiences cause a durable epigenetic modification of this gene [93]. In animal studies, it has been observed that variations in maternal care lead to a different methylation pattern of the GR gene with lasting effects on GR responsiveness in adults [94]. In humans, child abuse is linked to enhanced methylation of GR exon 1F promoter in leukocytes and in the postmortem hippocampus [50].

The early life experiences have a strong impact on gene expression and early life adversity can influence both GR and FKBP5 methylation. In PTSD, the GR promoter methylation induces an enhanced GR sensitivity with low levels of glucocorticoids, which decrease FKBP5 gene expression. This diminished FKBP5 gene expression could sustain the increased sensitivity of GR. Thus, the methylation of the FKBP5 promoter leads to an increase of this gene expression and, consequently, to a reduced GR sensitivity. Coincidently, patients who respond to treatment show a decrease methylation of the FKBP5 promoter and of GR sensitivity.

Methylation or demethylation of a number of other genes and their expression patterns are influenced by glucocorticoids. This includes genes linked to the synthesis of BDNF, which affects neurogenesis, of neuropeptides, such as neuropeptide Y(NPY) and α-MSH, or of enzymes involved in biosynthesis of neurohormones, such as the GABAergic neuroactive steroids [95–97].

2.3. The immune system modulation of stress responses

An inflammatory response characterized by increased number of granulocytes and monocytes, enhanced acute phase reactants and inflammatory cytokines levels has been demonstrated in individual with MDD compared to healthy controls [98–100]. Furthermore, the role of cytokines (interleukin IL-6, IL-1, and TNF-α) in the regulation of emotional behavior and memory formation during stressful life events, and their interaction with neurogenesis, glutamate and GABA signaling or changes in LTP has been documented [101,102].

The most promising inflammatory biomarkers in serum of subjects with psychiatric disorders are pentraxin C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), and IL-1 and IL-6 [103]. CRP, an acute-phase protein, produced by the liver, is found in plasma in response to inflammation and could be an important biomarker for psychiatric disorders, in addition to participating in inflammatory processes [104]. Indeed, an increase in CRP level was shown in patients with PTSD or in suicidal patients [105,106]. Some recent studies showed that patients with CRP levels above 10 mg/l responded better to treatment with TCAs or SSRIs than to psychotherapy alone [107] and that patients with low CRP levels (<1 mg/l) showed more consistent treatment response to escitalopram than to nortriptyline [108]. Taken together, these findings suggest that CRP may be an intriguing predictor of treatment outcomes that could lead to developing a personalized treatment approach. In depressed patients, an increased level of plasma CRP and IL-6 [109], and the serum or plasma levels of IL-1β and IL-6 are often related to antidepressant treatment, given that their levels inversely correlated with treatment response [110].

The investigation of mRNA levels of cytokines in MDD patients showed an increase of several inflammatory factors, such as IL-1β, IL-6, and TNF-α. Furthermore, two SNPs of IL-1β gene, rs16944 and rs1143643, were strongly associated with decreased responsiveness to antidepressants and the fMRI-analysis showed reduced amygdala hyperactivity to emotional stimuli [111]. A SNP was also discovered for the IL-6 gene, rs1800795, a potential diagnostic biomarker, which is associated with enhanced levels of plasma IL-6. Furthermore, its interaction with additional stressors increases the risk of MDD development or of other psychiatric disorders, such as schizophrenia [112,113]. A study by Sukoff Rizzo and colleagues, in rodents, demonstrated that an increased IL-6 level in the brain results in development of depressive-like behavior and the activation of IL-6 inhibits the antidepressant effect of SSRIs [114]. This finding may in part explain why some antidepressants fail to induce beneficial pharmacological effects in patients with MDD and PTSD.

Another cytokine that is increased in the plasma of depressed patients is TNF-α. Results show that failure to

normalize its levels also correlated with failed response to SSRIs [98,115]. In particular, recent studies suggest that responders to antidepressants show a lower expression of TNF-α. Lower expression of this cytokine (30%) was observed after successful treatment with escitalopram [116]. A meta-analysis study showed a specific profile in the changes of peripheral cytokines levels in MDD patients, which showed elevated concentration of IL-6, TNF-α, IL-10, sIL-2R, CCL-2, IL-13, IL-18, IL-12, and TNFR-2, while interferon-γ (IFN-γ) was slightly reduced [117]. A study by Goldsmith and colleagues [118] investigated the blood cytokine alterations in three different psychiatric disorders: schizophrenia, bipolar disorder, and depression. They found strong similarities among the changes in cytokine levels, raising the hypothesis of a common pathway for immune dysfunctions in these disorders. Interestingly, they also investigated the effects of antidepressant treatments on blood cytokines levels, showing also similarities in the changes of peripheral immune molecule levels. For example, in patients with MDD and schizophrenia, IL-6 decreases, while TNF-α fails to change.

Inflammatory responses play a pivotal role in PTSD as demonstrated by cytokinemia and meta-analysis studies revealed that PTSD patients have increased levels of TNF-α, IL-β and IL-6 in plasma [119]. Furthermore, PTSD risk and PTSD cases are associated with an increase of the expression of genes linked to innate immune responses mediated by interferon signaling. This evidence shows the importance of differences in innate immune factors for the development of PTSD [120]. A study by Pervanidou et al., showed an increased serum IL-6 concentration, measured 24 h after motor vehicle accidents, in children and adolescents who developed PTSD [121]. A longitudinal follow-up study revealed a normalization of IL-6 levels over time, validating the predictor role of IL-6 for PTSD. Moreover, other studies confirmed an increased IL-1β and IL-6 plasma levels and further showed that IL-1β is positively correlated to PTSD symptom duration, while IL-6 correlated to the PTSD symptom severity [119]. Contrarily, the proinflammatory cytokine IL-18 has been found downregulated in PTSD patients by a cDNA microarray investigation. The increased IL18 gene methylation explains the lower expression of this cytokine, which plays a neuroprotective role by inducing IFN-γ activity [122].

Whereas most PTSD studies have focused on the innate immune system, the acquired immune system can also be a mediator of risk and resilience of PTSD [123,124]. The regulatory T cells, such as CD4+ T, support CNS function, interacting with brain response to stress and promoting neuroprotection and neurodegeneration [125]. In chronic PTSD, there is a profound alteration of the composition of the peripheral T cell compartment [126]. Changes in T cell phenotype and increased differentiation of T cells are associated with PTSD and an elevated differentiation rate is taken as an early aging indicator of the immune system [127]. Combat veterans with PTSD showed a preponderance of CD4+ T helper 1 over the CD4+ T helper 2 cells, which is strongly correlated to enhanced IFN-γ plasma levels [128].

These findings collectively suggest that proinflammatory markers may be useful to predict treatment outcomes and for diagnosis of the pathology. Whether the enhancement of proinflammatory markers reflects a neural dysfunction or whether these molecules play a role in the pathogenesis of psychiatric disorders is unclear, however, their alteration may be valuable as a marker to determine the most appropriate treatment.

2.4. Neuroplasticity

PTSD and MDD are associated with low levels of neurotrophins, including BDNF, which play a fundamental role in neuronal growth, synaptic maturation and plasticity [129]. BDNF is fundamental for the development of the nervous system and decreased serum levels of this neurotrophin and/or of its receptor expression, TrkB, may be a useful predictor for dysfunctional behavior and in particular for suicidal ideations [105]. Studies in rodent models show a decreased BDNF mRNA expression in the hippocampus during chronic and acute stress, with consequent effects on hippocampal neurogenesis [130]. Furthermore, the reduction of BDNF mRNA expression has been associated with increased IL-6 expression [131]. Importantly, both glucocorticoids and proinflammatory cytokines have been often linked to a reduction of BDNF levels and to a diminished neurogenesis resulting in dendritic atrophy [132,133]. The mechanism by which proinflammatory cytokines could affect BDNF expression is still unclear. However, animal studies showed that IL-1β downregulates BDNF expression in rat hippocampus, probably by an indirect mechanism that relates to regulation of glucocorticoids [134]. Overall, depressed patients show a reduction of BDNF mRNA expression, with increased levels that correlated with symptom improvement in antidepressant responders [135]. A recent meta-analysis investigation has suggested that BDNF is a promising biomarker for MDD patients relevant to predict clinical improvement [136].

In contrast to the observations in MDD patients, the BDNF levels in the serum of subjects with PTSD are controversial. Some researchers reported that it increases in PTSD and it is even higher after traumatic events [137], while others showed lower levels [138]. Berger and colleagues found that serum BDNF level is a good predictor of the treatment outcome in PTSD: lower serum BDNF is a reliable marker for a successful treatment response to escitalopram in patients. The apparent discrepancy in BDNF results in PTSD patients may be explained by findings that the inhibition of BDNF activity could have mood-regulatory activity in some animal models, which suggests a biphasic concentration-dependent activity of BDNF [139]. The role of BDNF in the mesolimbic area is in contrast to the role of BDNF in the hippocampus, because high levels of BDNF in the dopamine pathway may be pivotal for the onset and maintenance of PTSD. BDNF, indeed, acts as a strong excitatory neurotransmitter, which evokes a rapid postsynaptic depolarization, promoting the dopamine release in the nucleus accumbens (NAc) [140]. In mice, the role of BDNF in this pathway is to maintain social avoidance behavior induced by the defeat stress paradigm. Hence, peripheral BDNF levels can provide an indicator of the activity of the dopaminergic system in the NAc and could be a predictor of poor response to treatment [139]. Furthermore, this discrepancy may also result from the methods used or differences in variables, including age or the subpopulation considered, all aspects that can alter the expression of BDNF and make the

interpretation of results difficult as well as limit the applicability of this potential biomarker.

Polymorphisms of the BDNF gene have also been investigated, the rs6265, resulting from a substitution of Val66Met, is frequently linked to a higher risk in developing affective disorders [141,142]. This SNP influences hippocampal volume and memory and may increase the susceptibility to both depression and PTSD [143]. Furthermore, the frequency of Val66Met is two times higher in PTSD patients than in healthy controls [144]. Stress and the Val66Met-allele seem to interact; a study on healthy European volunteers suggests that this interaction may result in development of depression and anxiety [145].

Whereas the association of BDNF levels and the symptoms of MDD and PTSD is still controversial, its role in the response to antidepressant treatment is clearer [146]. Several studies have demonstrated that chronic treatment with different SSRIs increases BDNF mRNA and protein expression in hippocampus and cerebral cortex [147,148]. Furthermore, all pharmacological classes of clinically used antidepressants increase TrkB autophosphorylation and signaling in hippocampus and forebrain [149,150]. Similarly, acute treatment with ketamine induces an increase in BDNF mRNA and TrkB phosphorylation [151]. Rodent studies show that BDNF and TrkB signaling is necessary for the behavioral effects of antidepressant drugs [146]. Indeed, reduction of BDNF levels in forebrain regions and inhibition of TrkB signaling in the dentate gyrus block the behavioral effects of antidepressants [150]. This evidence led to the hypothesis that antidepressants by stimulating neurotrophins promote neuronal plasticity and improve behavioral impairment in patients with MDD or PTSD [152]. In PTSD, the correlation between antidepressant effects and BDNF is more questionable, because few studies consider these three factors together. Berger et al. showed that despite an improvement of PTSD symptoms due to escitalopram, BDNF levels in blood failed to change [139]. In a case report of Hauck et al., PTSD patients, who were treated with sertraline for 6 weeks, showed a reduction of symptoms, but no increase in serum BDNF levels [153].

Measuring the levels of BDNF in blood may advance the understanding of the neurotrophins' role in mood disorders, whether it may offer a potential biomarker to monitor treatment response in PTSD patients remains unclear.

2.5. The NPY implications in resilience

NPY is an important mediator in the regulation of the stress responses [154]. NPY and its receptors play a fundamental role in decreasing fear, anxiety, and also improving memory processes [155,156]. Stress modulates NPY expression in the brain, but the duration and type of stress influence the magnitude and direction of NPY expression change [157]. Rodent studies showed that foot-shocks increase NPY gene expression while acute restraint stress exerts the opposite effect in the amygdala [158,159]. Moreover, acute but not chronic stress enhances NPY transcription levels in the hypothalamus, suggesting a possible habituation to repeated stress exposure [157]. NPY participates in the regulation of the HPA axis by interacting with the PVN of the hypothalamus and with the action of CRH [160]. Stimulation of NPY Y1 receptors in the hippocampus of rodents appears to inhibit the HPA axis, although some studies have reported different results [161–163]. For instance, the administration of the Y1 receptor antagonist, BIBP 3226 in the amygdala exerts an anxiogenic effect, while intracerebroventricular injections of a specific Y1 agonist ([D-His(26)]NPY) have an anxiolytic effect [126,164]. Another mechanism of action of NPY-induced behavioral changes emerged with the Y1 agonist (Leu^{31},Pro^{34}), which reduced the amplitude of NMDA-evoked excitatory postsynaptic currents and increases the inhibitory current magnitude induced by $GABA_A$ receptor activation [165].

The involvement of NPY in stress response and by improving emotional behavior is further analyzed in rodent stress models. Rats exposed to a predator scent stress protocol, an animal model of PTSD, showed that the animals with the lower NPY levels had the more significant behavioral disruption, and treatment with NPY reduced the altered behaviors [166]. The use of Y1 receptor antagonist, BIB03304 led to a behavioral disruption confirming the pivotal role of Y1 receptor in behavioral dysfunction [166]. Other animal studies have showed that NPY supplementation after trauma exposure prevents the development of PTSD-like behaviors. For example, the intranasal administration of NPY by increasing the concentration of NPY in the CNS, devoid of side effects, led to behavioral improvements in animal models for PTSD, such as the single prolonged stress and the predator exposure stress [167,168].

Evidence demonstrates that NPY is relevant in PTSD and MDD pathophysiology. Plasma NPY levels in humans increase after the exposure to acute stress. The enhancement of NPY concentration is positively correlated with the stress-induced increase of cortisol salivary levels [169]. During stress, the increased release of NPY may provide a positive psychological response, preventing the anxiogenic effect induced by CRH. Moreover, the peripheral increase of NPY detected in plasma may reach the brain and exert central effects. Interestingly, individuals who experienced dissociative PTSD symptoms, 1 week before stress exposure, showed a lower release of NPY in response to stress [169]. This evidence suggests that NPY may be a relevant biomarker to predict the ability of an individual to cope with stressful events. Veterans with PTSD showing symptoms of dissociation exhibit a reduced capacity to release NPY [170]. Thus, the altered release of NPY can influence the risk for the development of PTSD. Other studies showed that NPY concentrations in the CSF and plasma are reduced in patients with PTSD [170–172]. In particular, a study by Rasmusson et al. [170] found that basal and yohimbine-stimulated NPY plasma levels are lowered in PTSD subjects. Reduced NPY levels in plasma were found also in combat-exposed individual with PTSD [173]. However, NPY levels were also reduced in combat-exposed individuals without PTSD, showing that the trauma itself could be a factor, in this study, in the regulation of NPY concentrations [173]. However, later studies observed opposite results, showing both trauma-exposed PTSD patients with no alterations of NPY levels [174] and combat-exposed veterans without PTSD with a higher NPY expression [175]. Because of the questionable reliability of plasma NPY concentrations, other studies started to focus on its CSF levels. Sah and colleagues showed that combat-exposed veterans with PTSD have lower NPY levels in the CSF in comparison to trauma-exposed veterans without PTSD [172].

The abnormalities in NPY concentrations are of interest because NPY seems to be implicated in resilience development and prevents trauma-induced PTSD. Genetic evidence supports this role of NPY: Individuals with lower-haplotype NPY expression show exaggerated amygdala reactivity, which reflects an overreaction to stress [176]. However, direct associations between NPY gene polymorphisms with PTSD have not been observed. Several SNPs of the NPY gene have been studied and, for its role in stress responsiveness, rs16147 is a promising biomarker for vulnerability to anxiety and depressive symptoms [177]. The NPY SNP rs16147 is highly related to the variation of the NPY levels and is associated with a reduction of NPY expression [176]. Studies on young adults showed an interaction between rs16147 and early life adversity. In addition to rs16147, the polymorphisms rs3037354 and -1002T>G are important for the stress response. The SNP rs3037354 is associated with higher plasma NPY and changes in the expression of GR signaling [178]. On the contrary, loci in the NPY promoter (1002 T>G) induce a lower NPY expression detected in CSF, which is associated with hyperarousal during the exposure to stress [179].

Measurements of NPY in CSF of depressed patients led to overall divergent results. Several studies reported significant CSF NPY reduction in individuals with MDD, while other studies failed to find differences or showed higher NPY levels in CSF of depressed individuals [180]. The individual ability to adapt to stress and/or the genetic history may explain the differences found in NPY levels of MDD patients. Studies on NPY in individual with PTSD and comorbid MDD are under-investigated. Only a study in PTSD patients with comorbid depression by Sah et al. showed an inverse correlation between NPY concentration in CSF and PTSD diagnostic CAPS scores, but no correlations were found with Beck Depression Inventory (BDI) [172].

Altogether, whether NPY could be a valuable therapeutic target for PTSD and MDD and whether it may help facilitate resilience in vulnerable subjects remains a matter of debate [180,181].

2.6. The role of endocannabinoids and allopregnanolone in MDD and PTSD

2.6.1. The biosynthesis of neuroactive steroids

Neurosteroids and neuroactive steroids modulate neuronal functions after binding to nuclear intracellular receptors and by acting as transcription factors with important implications in modulation of gene expression [182]. Neuroactive steroids can also act via nongenomic mechanisms as potent modulators of ligand-gated ion channels, including $GABA_A$ and NMDA receptors [183,184]. Neuroactive steroids play a role in stress response, are implicated in neuropathology of an array of psychiatric disorders and may mediate the response to several psychotropic treatments [185].

Neurosteroids, allopregnanolone (Allo) and its stereoisomer, pregnanolone (PA), are synthesized in glutamatergic corticolimbic neurons, including cortical and hippocampal pyramidal neurons, and pyramidal-like neurons of the basolateral amygdala [186,187]. They rapidly modulate neuronal excitability by acting as potent positive allosteric modulators of the action of GABA at $GABA_A$ receptors and they are responsible for the fine-tuning of the receptor for GABAmimetic, agonists and positive allosteric modulators [188–191]. Recent finding in the field have suggested that the sulfated congeners of these neurosteroids, e.g. PA sulfate, act as inhibitors of tonic rather than phasic NMDA-mediated neurotransmission [192].

Downregulation of neurosteroid biosynthesis, which includes Allo and PA levels and their biosynthetic enzyme 5α-reductase type I and 3α-hydroxisteroid dehydrogenase (3α-HSD), is strongly associated with major depression and PTSD (for a review please see [193,194]). Specifically, patients with depression show serum, plasma, CSF, and brain reductions of Allo levels and/or biosynthesis, which may become important diagnostic biomarkers [195–198]. Likewise, depression and anxiety symptoms in both anorexic and obese females or during pregnancy and postpartum are associated with downregulated Allo levels [199,200]. The levels of Allo in the CSF are 40–60% decreased in patients with unipolar major depression and premenopausal women with PTSD [197]. The lowest levels were found in the PTSD patients with comorbid depression [201]. Altered Allo levels have been observed both in serum and CSF in several other neuropathologies, including postpartum depression and drug addiction [197,202]. Women with PTSD show lower Allo concentration in the CSF and serum, while progesterone and the immediate Allo precursor, 5α-DHP fail to change, pointing to a possible deficit in the enzyme 3α-HSD [203]. Likewise, in PTSD males, the CSF Allo levels decrease, probably because of deficits of 5α-reductase type 1, and are negatively correlated with PTSD symptoms [204]. Moreover, a SNP in the 5α-reductase type II gene is linked to enhanced risk for PTSD in men [205]. 5α-reductase type II is preferentially expressed in the periphery in the adrenal cortex [206]; however, peripheral GABAergic neuroactive steroids, including THDOC, are changed during stress and may access and influence corticolimbic circuitry [207]. Thus, the concentration and, in particular, the ratio of Allo with other neuroactive steroid levels and deficits in the enzymatic pathway may unveil sex-related biomarkers for PTSD and MDD, as well as provide a target for novel therapeutics to improve MDD and PTSD patients. Studies in depressed patients with low Allo concentrations in CSF and plasma showed that after SSRI treatment, increased Allo level correlated with improvement of depressive symptoms [196,197].

The plasma levels of the $GABA_A$ antagonist and neuroactive steroids, dehydroepiandrosterone (DHEA) and its sulfate derivatives, DHEA sulfate (DHEAS) are currently being investigated as potential biomarkers for anxiety, MDD, and PTSD. DHEA facilitated excitatory NMDA receptor function and plays a role in the inactivation of cortisol in its metabolite cortisone [208]. The DHEAS concentration and the ratio of DHEAS to cortisol predicts the severity of symptoms of PTSD and depression in patients [209]. In the CSF of women with PTSD, correlation between the ratio of DHEA to Allo levels and their symptoms was observed [201]. This suggests a role in the balance between excitatory and inhibitory neurotransmission and the severity of the pathology.

In mouse models of depression, induced by protracted social isolation stress, a ownregulation of Allo levels in corticolimbic neurons was observed and resulted from a decreased expression of 5α-reductase type I [210]. Furthermore, socially isolated

(SI) mice show increased aggression, anxiety-like behavior and exaggerated contextual fear responses [211,212]. In another model of PTSD, the single prolonged stress (SPS) mouse, downregulated cortical Allo levels were associated with enhanced anxiety-like behavior and enhanced contextual fear responses. Importantly, similarly as in PTSD patients [213], stress induces changes in $GABA_A$ receptor subunit composition in SI mice. Specifically, it increases expression of α4 and δ subunit that are: (1) mainly expressed in the extrasynaptic $GABA_A$ receptor; (2) show an increased sensitivity for neurosteroids; and, importantly, (3) fail to bind benzodiazepines, and therefore result in inefficacy to respond to their pharmacological action [202]. These findings are strikingly consistent with dysfunctions observed in PTSD patients. PET studies show PTSD patients have decreased benzodiazepine binding sites and lack of response to benzodiazepines [213]. Collectively, PTSD, like MDD, shows a downregulation of Allo biosynthesis; however, this and its interface with changes in $GABA_A$ receptor subunit expression and lack of response to benzodiazepines points to a biomarker axis (Figure 3), which may be specific for PTSD (discussed in [193]).

In SI mice administration of Allo or its analogs (ganaxolone, BR351, and BR297) reduces behavioral dysfunction [193,214]. Furthermore, SSRIs given at low non-serotonergic doses act as *selective brain steroidogenic stimulants* (SBSSs), upregulate Allo levels, and improve fear responses, anxiety-like behavior and aggression in Allo-deficient SI mice [215]. Consistently, in recent clinical trials, intravenous Allo (i.e. brexanolone; SAGE 547) or an orally active Allo's analog (SAGE 217) showed increased efficacy against symptoms of postpartum depression (PPD) and MDD compared to that of placebo [216]. PPD patients who received intravenous infusions of Allo showed a rapid and long lasting

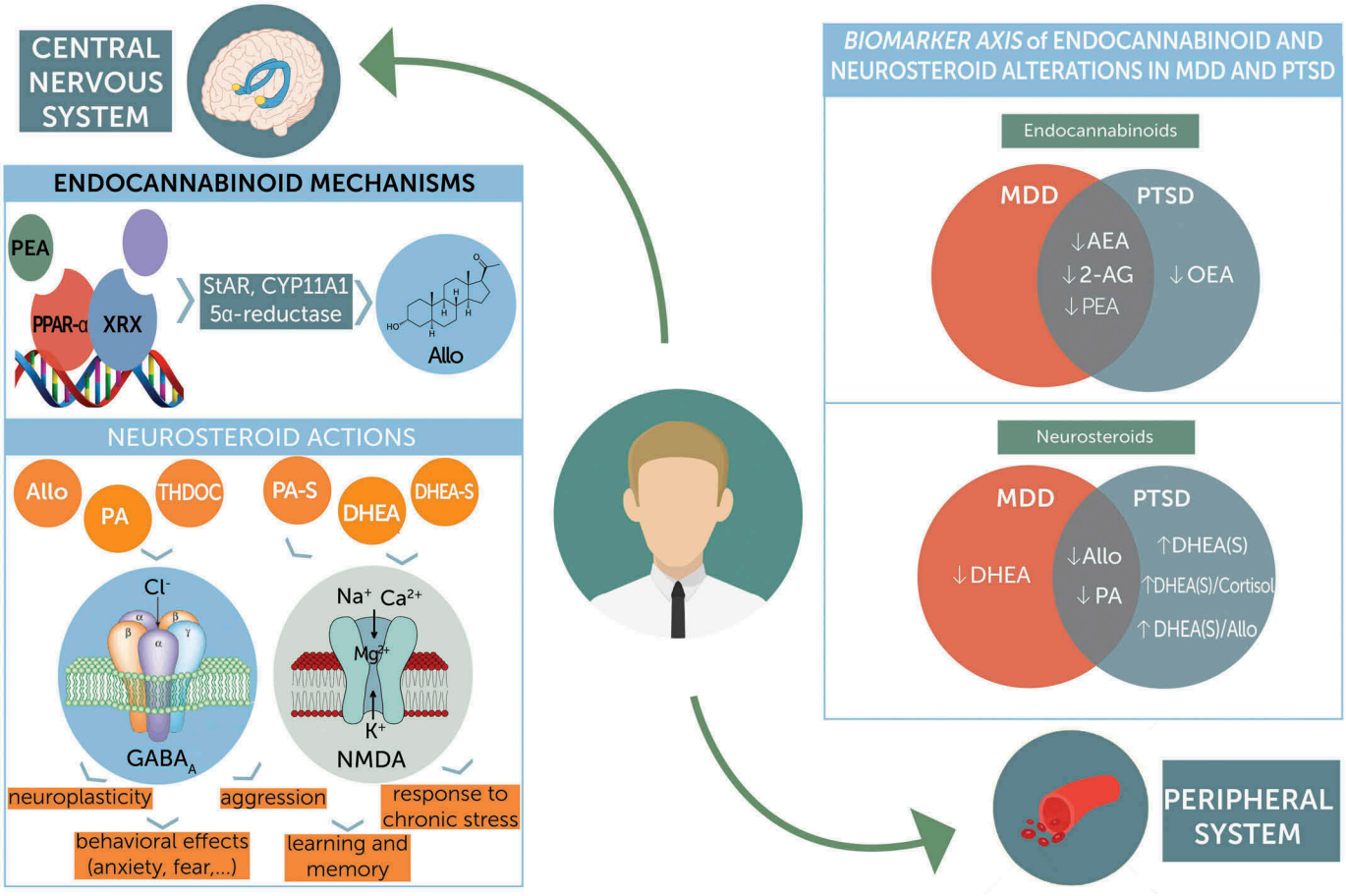

Figure 3. Potential role of endocannabinoids and neurosteroids as peripheral biomarkers for PTSD and MDD.

The schematic representation shows the mechanisms for the neurosteroidogenic action of the endocannabinoid-like, ethanolamides (e.g. PEA) in the brain (left panel). PEA and OEA activate the intracellular peroxisome proliferator-activated receptor (PPAR)-α, which heterodimerize with the retinoid X receptor (RXR). The PPAR-α and RXR complex binds to the consensus regions of target gene promoters and initiates transcription. PEA, through the activation of PPAR-α, can enhance the expression of corticolimbic allopregnanolone (Allo) biosynthetic enzymes, including CYP11A1 and 5α-reductase type I, resulting in an enhanced neurosteroid biosynthesis (e.g. Allo) [278]. Rodent studies confirmed that the administration of PEA or of PPAR-α agonists improve emotional behavior by increasing Allo levels [193]. PEA levels and probably expression of PPAR-α are influenced by stress, which may negatively affect Allo's biosynthetic enzyme expression and Allo levels. Allo and its stereoisomer pregnanolone (PA) are primarily synthesized in glutamatergic neurons and play a central neuromodulatory role in facilitating the action of GABA at $GABA_A$ receptors (a primary target of anxiolytics), and a role in the fine-tuning of the receptor for agonists and GABAmimetic agents. The finding that Allo facilitates the efficacy of $GABA_A$ receptor allosteric modulators substantiates its endogenous neurophysiological relevance. Allo and PA binding at $GABA_A$ receptors result in the improvement of behavioral responses, including anti-aggressive, anxiolytic, and anti-fear actions. Sulfated neurosteroids may act on NMDA receptors (bottom, left pannel). The binding of sulfated Allo and PA (PA-S) inhibits tonic-activated NMDA receptor neurotransmission, which results in important downstream effects on neuroplasticity, memory formation, and learning processes with a high relevance for neuroprotection and cognitive processes [192,193]. The altered levels of endocannabinoids, endocannabinoid congeners, and neurosteroids in the blood and/or CSF of PTSD and MDD patients are represented in the right panel. It is evident that the abnormal concentrations of endocannabinoids and neurosteroids in PTSD and MDD show common alterations. The discovery of specific dysfunctions in the peripheral tissue of individuals can provide biomarkers to diagnose and treat PTSD and MDD. Moreover, the relation of several biomarkers, by assessment of a biomarker axis, may help identify subpopulation of patients within one psychiatric disorder and may enable selection of individual-based therapeutic strategies.

remission of depressive symptoms in 70% of treated versus only 9% of placebo-treated patients.

Likewise, the 18kDa translocator protein (TSPO), which gates the entry of cholesterol from the cytosol into the inner mitochondrial membrane to initiate neurosteroidogenesis, resulted in a useful PTSD therapeutic target [217]. Drugs that act at TSPO stimulate downstream Allo levels in the brain of SPS mice and rescue behavioral dysfunctions [218]. By this mechanism, TSPO ligands promote anxiolytic effects in humans [218]. For example, the ligand, XBD173 potentiates GABA-mediated neurotransmission through the induction of neurosteroidogenesis [219]. XBD173 counteracts panic attacks in rodents and exerts anti-panic activity in humans [219]. Hence, TSPO drugs are fast-acting anxiolytics, devoid of tolerance liabilities and with fewer benzodiazepine-like side effects, such as tolerance, dependence, and withdrawal symptoms. Moreover, Ströhle and colleagues [220] showed an altered benzodiazepine-$GABA_A$ receptor function in patients with panic disorder, which suggests neurosteroid-based therapeutics that act on $GABA_A$ receptors may offer a treatment advantage over benzodiazepines for these patients [219]. In preclinical studies, the administration of the TSPO ligand, YL-IPA08, to rats subjected to chronic unpredictable stress (CUS) increased progesterone and Allo levels in the hippocampus and prefrontal cortex and induce antidepressant effects [221]. Moreover, the TSPO activation and the subsequent neurosteroid-induced synthesis contribute to maintain hippocampal morphologic and functional plasticity and to prevent the dysfunction of HPA axis observed in CUS rats [221]. After the administration of another TSPO ligand, etifoxine, rats showed a reduction of anxiety-like behavior, which correlated with increased Allo concentrations [222]. The administration of the 5α-reductase inhibitor, finasteride blocked etifoxine's pharmacological and behavioral effects, confirming that etifoxine acts by inducing neurosteroidogenesis [217]. Clinical studies have proven that etifoxine can improve anxiety-related disorders in humans [223]. Thus, TSPO ligands and other drugs that promote neurosteroidogenesis and increase Allo concentrations are promising therapeutic strategies for MDD and PTSD.

2.6.2. The endocannabinoid system

The endocannabinoid system is involved in HPA axis activation and its interaction with glucocorticoids is useful to cope with stress. Furthermore, the endocannabinoids modulate fear memory and other memory processes, like reconsolidation and extinction, which are a core feature of PTSD [224]. In a mouse model of depression, the hippocampal suppression of the endocannabinoid signaling leads to depressive-like behavior [225,226], thus suggesting it may play a role in PTSD and MDD.

The endocannabinoid receptor type 1 (CB1) has received growing attention in mood disorders. A positron emission tomography study showed enhanced expression in individuals with PTSD but not in trauma-exposed healthy controls [227]. Furthermore, this study found diminished peripheral level of the endocannabinoid, anandamide (AEA), which suggests that the enhanced CB1 receptor expression, and probably sensitivity, may be in part due to lower levels of AEA [227]. Likewise, a postmortem study revealed higher expression of CB_1 in depressed suicide victims [228]. However, genetic studies support the hypothesis that impairment in CB_1 may increase the risk to develop depression and other psychiatric pathologies. SNPs in the CB_1 gene can increase the vulnerability to develop depressive episode after trauma exposure and in patients with mood disorders when the frequency of SNPs increases [229,230]. Moreover, AEA and the congener, 2-arachidonoylglycerol (2-AG) serum concentration is lower in the plasma of depressed women than in matched control subjects [231]. The levels of these endocannabinoids in depressed women are a focus of investigation and several considerations of whether they may be valuable markers are being discussed. Likewise, rodent models of PTSD and depression show decreased levels of AEA and 2-AG [232]. For example, in CUS rats, the number of CB1 receptor binding sites increase, while the levels of AEA decrease in the prefrontal cortex, ventral striatum, and hippocampus [233]. The administration of AM404, an endocannabinoid reuptake blocker, facilitates fear extinction in rats by enhancing AEA and 2-AG concentrations [234]. This evidence confirms the relevance of AEA levels to facilitate fear extinction in the basolateral amygdala [235]. In human studies, AEA concentrations are reduced in PTSD patients compared both to healthy controls and to trauma-exposed controls [227].

Other endocannabinoid-like molecules that activate the peroxisome proliferator-activated receptor-alpha (PPAR-α), such as N-oleoyld-opamine (OEA) and N-palmitoylethanolamine (PEA) may be involved in the pathophysiology of PTSD and MDD [236]. PPAR-α activation has been shown to mediate the response to stressful conditions [237]. In healthy adults, PEA significantly increases in clinical stress tests in connection with an increase of cortisol levels [238]. PEA levels also decrease when healthy subjects experience a short-term depressed mood [239]. On the other hand, PEA levels in PTSD, MDD, and impulsive aggression are decreased [240]. Of note, PEA adjunctive therapy to citalopram improves depressed symptoms [241] and intense physical activity increases PEA and OEA levels while improving depression and PTSD symptoms [242]. The relationship between PPAR-α and emotional regulation is further highlighted by its role as an anti-neuroinflammatory target [243–247]. In recent studies originated in our laboratory we have shown that stimulation of PPAR-α by PEA or synthetic agonists increased corticolimbic Allo levels in hippocampus, amygdala, and prefrontal cortex, which facilitated contextual fear extinction and fear extinction retention, and improved aggression and anxiety-like behavior in SI mice [193,248]. PEA also induces antidepressant-like effects in SI mice [193]. Furthermore, this behavioral improvement by PEA or other PPAR-α synthetic agonists, which was associated with normalization of Allo levels, was blunted by antagonism at PPAR-α, inhibition of Allo biosynthetic enzymes, and in PPAR-α KO mice. Other rodent studies have reported that exposure to predator stressors downregulates PEA and OEA levels [249], but treatments that restore PEA and OEA levels result in antidepressant-like effects [250–252].

AEA and other CB1 endogenous agonists are of interest as biomarkers in PTSD and MDD diagnose and possibly as endpoints of treatment outcome. Furthermore, preclinical and clinical studies support an emerging role of PPAR-α in MDD and PTSD. The discovery of this new interrelation between

PPAR-α activation and Allo biosynthesis may unveil a *biomarker axis* uniquely altered at the interface of the endocannabinoid and the neurosteroid systems (Figure 3).

3. Blood-based biomarker axis

3.1. The potential of peripheral biomarkers

All biomarkers discussed in this review article have been studied in animal models or in humans, with a focus on the CNS and CSF, serum, and plasma [253,254]. Noninvasive peripheral biomarkers are more useful and functional for diagnosis of disorders of mood and emotions. CSF reflects more closely the alterations of the brain, but the procedure is stressful and results in pain and discomfort in the patients [255,256]. Blood draws can also be stressful for some patients, putting at risk a precise diagnosis or may even turn patients away from testing. However, most studies are currently assessing serum- or plasma-based diagnostic tests [257], considering different biomarkers and several methods, including metabolomics (neurohormones, neuroactive steroids, endocannabinoids) or genomics and proteomics. The majority of techniques used for proteomic analyses are based on the combination of two-dimensional gel electrophoresis (2DE) to separate proteins and mass spectrometry (MS) for their identification. The main advantage of this technique consists in the quantification of several biomarkers simultaneously in small sample amounts; while, the disadvantages include very low reproducibility and the need of targeted analyses to discover a potential biomarker. Despite the power to separate several proteins simultaneously, the 2DE-MS technique is limited by the difficulty in detecting low-abundance proteins and proteins with extremely high or low molecular weight [258]. An alternative MS-based approach is shotgun proteomics or liquid chromatography-tandem mass spectrometry (LC-MS/MS), which provides several chromatographic steps prior to MS analyses. Shotgun proteomics has the advantage of being more sensitive in detecting differences in proteins expressed in patients with MDD [259], as well as alterations in protein expression in patients with schizophrenia, which were not detected by using 2DE-MS [260]. Validation of the potential biomarkers are additionally assessed by employing other methods, such as Western blot, or the enzyme-linked immune-adsorbent assay (ELISA) [261].

Another valuable proteomic method, which allows to simultaneously measuring multiple proteins in a small volume of sample, is the Luminex bead-based approach. This technique has been successfully used for biomarker discovery and is more common in several clinical studies such as in studies of autoimmune disorders [262], or Alzheimer's disease [263]. Several other techniques involve the use of stable isotopes to label peptides. The most widely known is the Isotope Coded Affinity Tags, which permits to distinguish between different protein in one sample based on their isotopic composition, separating the peptide fragments of interest and quantifying them by MS [264]. By labeling only cysteine residues, this approach only analyzes proteins containing this amino-acid residue, hence excluding many other relevant proteins.

More recently, the development of the isobar tags for relative and absolute quantification (iTRAQ) technology improved the quality of protein identification and quantification, reducing the assay variations observed in multiple MS quantification [265]. The combined use of iTRAQ and LC-MS /MS is a promising technology for protein identification [266]. The iTRAQ-LC-MS/MS technology is one of the most sensitive proteomic assays that can quantitatively analyze low-abundance proteins in biological samples [267]. This approach has been employed in different disorders, including cardiovascular disease, neurological, and psychiatric disorders [266,268,269]. Interestingly, the iTRAQ approach enables the identification of human peripheral serum protein expression that may offer relevant biomarkers for the diagnosis of depression [270] and allows distinguishing between MDD and other psychiatric disorders, such as bipolar disorder [271]. The iTRAQ methodology detected differences in serum expression between depressed patients and healthy controls and ELISA confirmed a significant increase of CRP, ITIH4, SAA1, and ANGPTL3 protein levels in patients with clinical depression compared to healthy controls. Thus, these encouraging results suggest that iTRAQ is a useful methodological tool to identify candidate biomarkers in serum from patients. For the routine evaluation of protein levels, ELISA tests are more practical and time effective.

In our laboratory, with the goal of assessing biomarkers, we employ the gas chromatography-mass spectrometry (GC-MS) to determine neuroactive steroids in CSF, serum, and plasma of MDD and PTSD patients but also in serum and discrete brain regions of mouse models of these disorders. Our results have identified specific changes in the axis of neuroactive steroid biosynthesis and their relation with neurotransmitter systems, including $GABA_A$ receptors [193] (Figure 3 and Figure 5). While the GC-MS technique offers a powerful tool in biomarker discovery, the feasibility of using the GC-MS technology as a routine test to evaluate biomarker concentrations in tissue of patients and individuals at risk is limited by its costs, complexity and it is time-consuming. However, once firmly established and structure selectivity achieved, the biomarkers' evaluation may be substituted, in a later stage, by ELISA determinations to satisfy the market time and cost requirements.

In addition, gene-activity assay is a promising technique and cost-efficient; however, this technology requires more investigation to identify specific genes that change their expression in PTSD and MDD [88]. DNA microarrays can efficiently highlight gene expression profiling of transcriptional reactivity. Currently, studies that analyze gene expression related to PTSD showed relevant signatures in mononuclear cells that may be useful to diagnose a mental disorder [272]. However, improved methods are still required to screen more efficiently through sets of candidate variants, and then, a rigorous validation of variants and gene effects are also needed [273]. Furthermore, replications in larger samples and investigations focusing on selected markers as part of the biosignatures that have been discovered, are required to assess the diagnostic utility and pathological relevance of these methods.

Several experimental protocols that consider not one or a few, but several biomarkers are advantageous and have been recently proposed [261,274]. These protocols provide the evaluation of many biomarkers together. For example, Papakostas

and colleagues proposed a diagnostic test based on determining neuropeptides that provides an adequate sensitivity to distinguish MDD from nondepressed subjects [275]. Other studies considered a panel of blood transcriptomic biomarkers to predict early onset MDD [276] and to identify depressed patients in remission or predict response to therapy [274]. Transcriptomic-based biomarkers, including FAM46A, MARCKS, and RAPH1 appear to be particular promising. Evaluation of blood-based tests also showed that candidate biomarker transcripts are promising for psychiatric disorders [274,276–278]. Also, functional genomics tests have been proposed, which analyze genes involved in several functions from myelination to growth factor signaling [261].

A consistent number of studies are currently assessing promising biomarker tests for MDD and PTSD to provide a preclinical screening, a precise and accurate analytical validation of the marker and a clinical validation. These criteria may lead to standardized methods and establishing interrelations among peripheral biomarkers for a reliable diagnosis of these psychiatric disorders. Correlative studies among potential biomarker candidates and saliva should ascertain the reliability for diagnosis and when possible, a saliva test should be preferred.

The process for a test-assessment based on biomarkers, from discovery to validation, is schematized in Figure 4.

4. Expert commentary

4.1. The advantage of a 'biomarker axis' in diagnosing, predicting, and treating MDD and PTSD

MDD and PTSD are heterogeneous, complex, and debilitating neuropathologies that share several neurochemical abnormalities and, therefore, it is not conceivable that a single alteration could point to a specific disorder. Furthermore, the frequent overlap of symptoms and alterations between MDD, PTSD, anxiety disorder, and suicidal ideations makes it unrealistic to consider only a few biomarkers to identify each disorder. Identifying biomarkers is also fundamental for early detection of MDD and PTSD and rapid intervention. Currently, the trend in the field is leading toward developing individually based therapies rather than one-size-fits-all treatments, which is the old-school approach currently being used with the prescription of SSRIs. However, the inefficiency of SSRIs prompted the need of a clearer understanding of the mechanisms underlying the MDD and

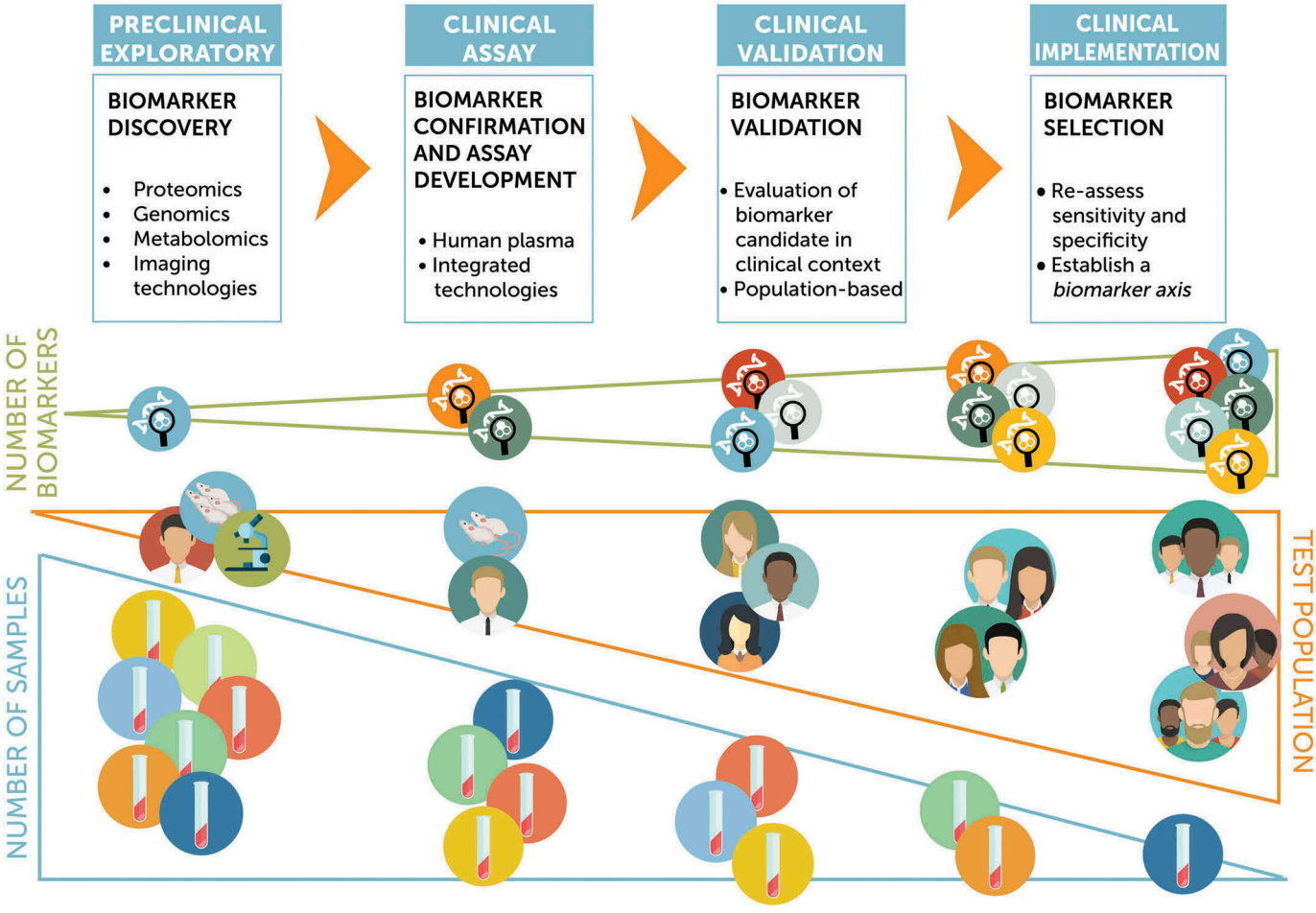

Figure 4. Process of developing a biomarker-based assessment of psychiatric disorders.

The process that leads to the selection of biomarkers that are useful to predict, diagnose, and treat psychiatric disorders or test individual susceptibility is articulated in several phases. Discovery for potential biomarkers, mainly on animal models, is a long process that requires validation on human samples through different sophisticated technologies; such as gas chromatography-mass spectrometry (GC-MS) alone or combined with imagine techniques or proteomics. Once the biomarkers have been established in preclinical studies, they need to be validated by clinical procedures that are applied to large multisite clinical studies. After this phase is completed, the biomarkers that have been selected will go through clinical implementation that improves the specificity and sensitivity of the markers. When more biomarkers are validated, a higher disorder-specificity can be achieved by identifying a *biomarker axis* that allows a precise diagnosis and selection of individualized treatments.

PTSD neurobiology. Appropriate treatments can improve the patients' quality of life, reduce the costs of inappropriate drugs, and facilitate a rapid recovery from the pathology. Blood-based tests are moderately invasive, practical, reliable and objective as opposed to self-reported diagnosis. As a more appropriate approach, we propose to establish a specific *biomarker axis* in which the synergistic effects of various biomarkers underline the constant interrelation between them in one disorder. For example, a downregulated content of a neuroactive steroid could lead to a 'domino effect', changing the expression or function of neurotransmitter system, including $GABA_A$ receptor subunit subtypes (discussed in [193]). A single biomarker by itself can have a very small impact on the neuropathology examined, but when incorporated in the overall picture of more neurochemical alterations, the relevance and the exclusiveness of all those changes together may become relevant and specific. Thus, establishing the relation of various biomarkers among each other, or in other words assessing a *biomarker axis* may be useful to distinguish different disorders, and differentiate, within one psychiatric disorder, various subpopulations that present specific comorbidities or characteristic behavioral traits. We anticipate that this approach will be very powerful in carrying out targeted interventions in the subpopulation under consideration. Knowledge of the primary neurochemical alterations in a patient allows clinicians to use the most effective treatments. For example, it allows understanding in which situation drugs such as an SSRI should be avoided and when an intranasal oxytocin administration should instead be preferred. It may also help decide if administering a combination of drugs with, possibly, synergistic effects is more appropriate to enhance the patient's recovery.

Hence, establishing a *biomarker axis*, which univocally identifies profiles of altered biomarkers in a specific disorder offers undoubted benefits. For example, as reviewed earlier, in PTSD, the decreased Allo biosynthesis, the changes in $GABA_A$ receptor subunit expression, and the lack of benzodiazepine pharmacological effects form a peculiar profile of PTSD and are essential to identify a potential PTSD *biomarker axis*. Furthermore, the various neurochemical alterations at the interface of PPAR-α and Allo biosynthesis may provide additional biomarker candidates for a more efficient diagnosis and therapeutic approach of patients with PTSD [279,280]. A schematic representation of a *biomarker axis* that we proposed for PTSD incorporating changes in the *PPAR-Allo axis* is depicted in Figure 5.

The role of the environment in the onset of MDD and PTSD is crucial and the predictive power of biomarkers is pivotal to anticipate the occurrence of the disorder. Predicting the onset of a specific disorder is particularly important for children and adolescents at risk so to provide timely support and minimize the possibility of developing a more severe disorder. Thus, assessing a predictive *biomarker axis* in young individuals may enable a timely use of preventive strategies. Finally, the assessment of an appropriate *biomarker axis* to diagnose MDD and PTSD will be fundamental in establishing an individually based diagnosis and therapy that takes into consideration the several facets of each subpopulation in one disorder. As a matter of fact, different subpopulations within one disorder can also be uniquely identified by assessing the interrelation of various biomarkers. New techniques will allow for a quicker and reliable characterization of the peripheral *biomarker axis* for accurate diagnosis and appropriate treatment.

Knowledge of family history, genetic polymorphisms, and environmental factors, such as stress and trauma exposure or substance abuse alters consistently the incidence of, and can be important predictors for MDD and PTSD. Thus, demographic and environmental factors should be considered together with blood-based biomarker analysis during the process of diagnosis and treatment assessment. When possible, a saliva test offers a better approach rather than a blood test in PTSD and MDD diagnosis.

Nonetheless the initial lack of progress in the field, current investment and research suggest that finding robust and reliable biomarkers is on the horizon. The advantage of relying on objective diagnosis and more appropriate treatment selections as well as the possibility of predicting susceptibility to MDD and PTSD will modify its incidence in an unexpected way.

5. Five-year view

Research on reliable biomarkers in psychiatric disorders has been considerably intensified in past years. More sophisticated technology, such as fMRI imaging or the GC-MS will lead to a greater chance to discover fundamental neurobiological alterations in MDD and PTSD. In the next years, the discovery of novel potential biomarkers and the increasing knowledge of neurochemical mechanisms in PTSD and MDD will provide a more accurate understanding of their role in the pathophysiology of these disorders and their diagnostic reliability through blood tests. Preclinical research will still remain an essential player in the effort to discovering new brain alterations. Animal-based studies are a primary requirement to identify promising peripheral biomarkers and relating them with behavioral changes observed in models of PTSD and MDD. We predict that the assessments of a *biomarker axis* for a specific disorder will likely be instrumental in paving the way for future test development. This will require strategic planning that includes the analysis of several biomarkers using larger populations and the development of more and more sophisticated techniques.

Thorough libraries that consider the demographic information for each individual and the type of trauma they were exposed to (e.g. combat exposure vs. domestic abuse, etc.,) will refine the predictive validity in the assessment of the diagnosis. This will tremendously impact testing new drugs by designing clinical trials based on selected subpopulations that show specific neurochemical alteration combined with factors including ethnicity, sex, age, genetics, environmental factors, etc. Such stratified clinical trials aim at normalizing the specific target for which the new drug was developed. This will increase the odds of a successful clinical trial and speed up drug development. The assessment of a relationship between: (1) validity of peripheral biomarkers per behavioral deficit, and (2) drug efficacy per deficient target will empower prediction, diagnosis, and treatment for the benefit of depressed or PTSD patient.

We trust that the next 5 years will bring a complete transformation of how we conceive mental health, along with diminishing the psychiatric disorder-associated stigma. By providing blood/saliva tests for PTSD and MDD, we hope

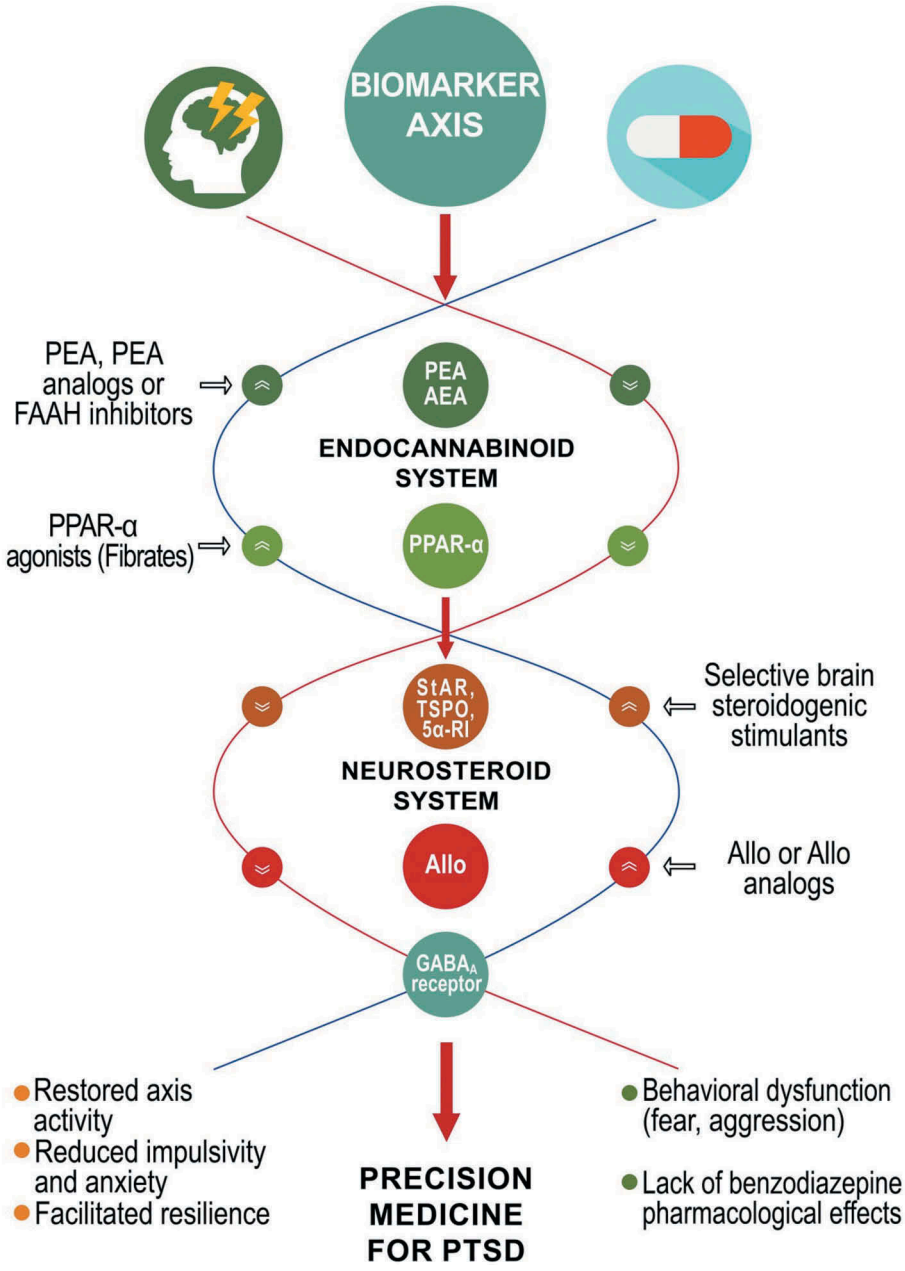

Figure 5. A proposed *biomarker axis* for PTSD at the interface of the endocannabinoid and neurosteroid systems.

Rodent models of PTSD have showed behavioral dysfunctions, including increased fear responses, aggression and anxiety-like behavior that correlate to decreased corticolimbic allopregnanolone (Allo) levels. Prolonged stressors induce a downregulation of Allo biosynthetic enzymes, such as the rate limiting-step enzyme, 5α-reductase type I, 5α-RI [215]. Furthermore, stress may alter $GABA_A$ receptor subunit expression, including increased α4, α5, and δ subunits and decreased α1, α2, and γ2 subunit expression [reviewed in 193]. The synaptic $GABA_A$ receptors composed by the α, β, and γ subunits mediate inhibitory phasic currents and they are highly sensitive to benzodiazepines. However, these receptors show a reduced sensitivity to GABA and Allo [281,282,]. Conversely, the extrasynaptic $GABA_A$ receptors, which are composed by α, β, and δ subunits, are responsible for the inhibitory tonic currents. These receptors are particularly sensitive to Allo, but not to benzodiazepines [283]. Thus, the alteration of $GABA_A$ receptor subunit composition induced by stress leads to receptor configurations with higher sensitivity for Allo and its analogs. This change of $GABA_A$ receptor composition decreases brain benzodiazepine binding sites resulting in a lack of benzodiazepine pharmacological effects [279]. The decreased Allo biosynthesis and decreased benzodiazepine binding sites have also been reported in PTSD patients [213]. Therapeutic approaches targeting Allo downregulation, such as the use of selective brain steroidogenic stimulants (SBSSs) or using Allo analogs are promising strategies for PTSD treatments [279]. Stress can also affect the endocannabinoid system by decreasing PEA and AEA concentrations and may impair the behavioral response. The activation of PPAR-α by endocannabinoids (AEA) and endocannabinoid-like molecules (PEA) may represent a mechanism through which these neuromodulators regulate stress responses [284]. By enhancing PPAR-α function, PEA or other synthetic PPAR-α agonists induce the biosynthesis of Allo in hippocampus, amygdala, and frontal cortex [279]. This is associated with behavioral improvement in rodent model of PTSD, such as the SI mice [193]. The stress-induced alterations at the interface of the endocannabinoid and neurosteroid systems, which includes the dysfunction of the endocannabinoids (e.g. AEA), endocannabinoid congeners (PEA), and neurosteroids (Allo) levels and biosynthetic enzymes, and/or the altered expression of their receptors PPAR-α, $GABA_A$, and NMDA receptors, may provide an important *biomarker axis* to selectively predict, diagnose, and establish the best individualized treatment selection for PTSD patients.

it will help both individuals who are currently in need but do not seek treatment and the approval of more successful medications for a larger number of patients.

Key issues

- MDD and PTSD are heterogeneous and complex psychiatric disorders with overlapping symptoms. The diagnosis of these neuropathologies is based on the evaluation of self-reported symptoms, leading to frequent misdiagnosis or underdiagnosis.
- The most used treatment, the SSRIs, proved ineffective for about half of the treatment-seeking patients [285]. This evidence suggests the need to develop targeted individually based treatments.
- Biomarker research is rapidly evolving. The identification of neuronal markers that reflect the behavioral alteration observed in MDD and PTSD is currently under intense investigation and promises to change diagnostic and treatment approaches.
- In addition to the well-characterized modification of the HPA axis, assessment of peripheral alterations that reflect brain changes, such as the proinflammatory, endocannabinoid and the neuroactive steroid levels in MDD and PTSD will lead to novel biomarkers.
- A novel approach to appropriately diagnose MDD and PTSD may be the consideration of a *biomarker axis* in which the synergic effects of several biomarkers interrelate. A biomarker axis may help identify a disorder, but may also lead to identifying different subpopulation within the same psychiatric disorder and selecting the best individualized treatment.
- Establishing a blood-based test for PTSD and MDD may not be a far reach, but more investigation is still required. Peripheral data analysis combined with knowledge of a patient's environment and family history will make it a quicker process.

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Declaration of interest

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