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New translational perspectives for blood-based biomarkers of PTSD: From glucocorticoid to immune mediators of stress susceptibility

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Abstract

Although biological systems have evolved to promote stress-resilience, there is variation in stress-responses. Understanding the biological basis of such individual differences has implications for understanding Posttraumatic Stress Disorder (PTSD) etiology, which is a maladaptive response to trauma occurring only in a subset of vulnerable individuals. PTSD involves failure to reinstate physiological homeostasis after traumatic events and is due to either intrinsic or trauma-related alterations in physiological systems across the body. Master homeostatic regulators that circulate and operate throughout the organism, such as stress hormones (e.g., glucocorticoids) and immune mediators (e.g., cytokines), are at the crossroads of peripheral and central susceptibility pathways and represent promising functional biomarkers of stress-response and target for novel therapeutics.

Keywords

PTSD; stress; individual differences; glucocorticoids; immune system; biomarkers; novel treatments

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Supplementary Information accompanies the paper

Introduction

While there has been progress in identifying blood-based biological perturbations, such as dysregulation of the HPA-axis and the immune system that are associated with vulnerability to stress-related mental disorders (Michopoulos et al., 2015; Pitman et al., 2012; Yehuda et al., 2015d; Zoladz and Diamond, 2013), it has been difficult to identify reliable blood biomarkers for use as diagnostic tools. The development of new system-wide techniques (i.e., genomic, epigenomic, transcriptomic, proteomic and metabolomic profiling) has permitted a greater examination of potential drivers or functional outcomes of stress-related dysregulation, advancing unbiased discovery of novel biomarkers implicated in PTSD susceptibility. This paper demonstrates how peripheral biomarkers, identified by candidate-based or genome-wide approaches, can be validated and developed further for *in vitro* and *in vivo* experimental models.

We briefly review HPA-axis' and immune system's involvement in PTSD and describe two major limitations that can be addressed by prospective studies: (i) whether they represent risk factors; and (ii) whether the two systems are interlinked. In the extent they represent risk factors, this justifies examination of individual differences in animal models of PTSD. We describe data from two animal models validating that these variables are risk factors. Finally we discuss clinical implications of the described findings for diagnosis and treatment.

Glucocorticoids (GCs) and peripheral inflammation in PTSD

Converging data have identified the hypothalamic-pituitary-adrenal (HPA) axis functioning as relevant to understand the pathophysiology of stress-related psychiatric disorders. The rationale for examining this system was clear: it is the main coordinator of the neuroendocrine stress response (de Kloet et al., 2005). Although numerous changes in HPA-axis (re)activity have been observed in PTSD, some of the findings have not followed those described as part of classic acute or chronic responses to challenge. GC alterations in PTSD [recently reviewed in: (Yehuda et al., 2015d)] involve:

i. Altered adrenal production of GCs. PTSD has been mostly associated with lower levels of the stress hormone cortisol (Yehuda et al., 2015d). However, some studies failed to observe differences in the basal GC tone and others reported increased basal cortisol levels (Klaassens et al., 2012; Meewisse et al., 2007; Morris et al., 2012). Various explanations have been proposed for these discrepancies such as trauma history, sampling conditions, diurnal rhythm, age, gender, comorbid factors etc.

ii. Alterations in tissue sensitivity to exogenous GCs. Heightened sensitivity to GCs was observed in peripheral blood mononuclear cells (PBMCs), by measuring the dexamethasone (DEX) induced inhibition of lysozyme activity ($IC_{50\text{-}DEX}$) as part of the non-specific immune response (Yehuda et al., 2004b). In addition, lipopolysaccharide (LPS)-induced cytokine production from leukocytes, a measure of monocyte responsiveness, has been reported to be more sensitive to DEX in samples collected from PTSD patients compared to samples collected from controls (Rohleder et

al., 2004). GC effects on T-cell functioning have also been examined in PTSD patients. Actually, decreased GC sensitivity of phytohaemagglutinin (PHA)-induced T-cell proliferation has been observed (de Kloet et al., 2007a). Taken together, this suggests that PTSD involves increased GC sensitivity in some immune cell-types and decreased GC sensitivity in others or even that there are pathways within the same cell-type with enhanced GC sensitivity and other pathways with reduced GC sensitivity. Suppression of endogenous cortisol by oral administration of low dose of DEX, a measure of the HPA-axis GC negative feedback at the pituitary level that correlates with the PBMC lysozyme $IC_{50\text{-}DEX}$ (Yehuda et al., 2003), is also enhanced in PTSD (Stein et al., 1997; Yehuda et al., 2004a; Yehuda et al., 1993).

Despite the fact that the mechanisms leading to HPA-axis dysregulation in PTSD are not fully elucidated, others and we have recently observed genetic variants and epigenetic differences within the *NR3C1* gene encoding the **GC receptor (GR)**, (Bachmann et al., 2005; Yehuda et al., 2015c). These differences have been proven functional at the gene expression or downstream neuroendocrinology levels in assays using primarily peripheral tissue (Daskalakis and Yehuda, 2014b), but it is expected that they have body-wide effects, as GR operates as a transcription factor across the body (Daskalakis et al., 2014). Functional genetic and epigenetic differences have been described also for genes encoding GR-chaperone proteins such as FK506-binding protein 5 (FKBP5), a GR-binding inhibitor, and spindle and kinetochore-associated protein 2 (SKA2), which is involved in GR-translocation to the nucleus (Binder et al., 2008; Boks et al., 2016; Kaminsky et al., 2015; Klengel et al., 2013; Sadeh et al., 2016). Interestingly, some of the observed traumatic stress- and/or PTSD-induced epigenetic effects could be intergenerationally transmitted (Daskalakis and Yehuda, 2014b; Perroud et al., 2014; Yehuda et al., 2015b; Yehuda et al., 2014a). Finally, expression profiling studies have detected altered peripheral blood expression of many genes of the GR signaling pathway in PTSD (Logue et al., 2015; Mehta et al., 2011; Yehuda et al., 2009) confirming the candidate gene studies.

Furthermore, in interaction with the genetic background (hit-1), early-life events or experiences (hit-2) prior to a traumatic event (hit-3) can influence PTSD-risk or -resilience (Daskalakis et al., 2013a). Thus, it is possible that life events that, by themselves, do not lead to psychopathology, in interaction with genetic predisposition lead to the development of a neurobehavioral substrate (e.g., HPA-axis dysregulation) that is more vulnerable to a traumatic event. For instance, polymorphisms in *FKBP5* gene interact with early trauma or childhood abuse to predict adult PTSD (Zannas and Binder, 2014). Mechanistically, there is an allele-specific, early trauma exposure–dependent peripheral leukocyte demethylation of 5'—Cytosine—phosphate—Guanine—3' sites (CpGs) close to and within glucocorticoid response elements (GREs) in intron 7 of *FKBP5*. Cytosine methylation in intron 7 is functional as demonstrated by the dexamethasone-mediated inhibition of LPS-induced interleukin 6 (IL-6) production in peripheral blood monocytes *ex vivo* (Klengel et al., 2013).

By definition, stress-sensitive genes are expressed in stress-sensitive systems (sympathetic nervous system, corticotropin-releasing hormone system, immune system) and are designed

to respond transcriptionally to high nuclear levels of GC-bound GR. In PTSD, due to low GC tone these GR-mediated transcriptional responses of stress-sensitive genes are compromised, resulting in a lack of recovery of the stress-induced alterations (Daskalakis et al., 2013b; Raison and Miller, 2003). In line with this notion, patients with PTSD have higher levels of multiple inflammatory proteins; the first meta-analysis indicated that **IFN-γ, interleukin 1β (IL-1β), IL-6** and **tumor necrosis factor α (TNF-α)** are the most consistently elevated pro-inflammatory cytokines in the blood of patients with PTSD compared with healthy controls (Passos et al., 2015). Moreover, unbiased genome-wide analysis found altered DNA methylation or RNA expression in multiple genes related to immune function in subjects with PTSD (Neylan et al., 2011; O'Donovan et al., 2011; Smith et al., 2011; Uddin et al., 2010).

Findings from prospective studies

The HPA-axis and the immune system are interconnected (Hodes et al., 2015; Miller and Raison, 2016; Raison and Miller, 2003), but we don't really know how they regulate each other in PTSD because they have not been examined together longitudinally. To overcome this, prospective sampling and clinical assessment are needed before and after a traumatic experience. To date two large prospective studies in the military have studied these markers at pre- and post-deployment: the Dutch Prospective Research in Stress related Military Operations (PRISMO) study and the U.S. Marine Resiliency Studies (MRS, MRS-II). The common, potential limitation of the studies is the low post-deployment rate of PTSD (Breen et al., 2015; Minassian et al., 2015; Reijnen et al., 2015).

Findings from the Dutch PRISMO study revealed that pre-deployment PBMC GR signaling pathway components (more glucocorticoid binding sites, low *FKBP5* mRNA expression, and high *GILZ* mRNA expression), but not LPS- or T-cell-mitogen-induced cytokine production, predicted the development of more intense PTSD symptoms post-deployment (van Zuiden et al., 2012; van Zuiden et al., 2011a; van Zuiden et al., 2011b). However, both high mitogen-stimulated T-cell cytokine production and high innate cytokine production were associated with increases in PTSD symptoms in response to post-deployment severe life events (Smid et al., 2015).

In a report from the MRS peripheral inflammation was reported to be predictive of PTSD development. Pre-deployment higher plasma concentrations of C-reactive protein (CRP), a marker of peripheral inflammation, were predictive of PTSD emergence (Eraly et al., 2014). No GC-related measures have as yet been reported from the MRS study.

Master regulators

Gene expression profiling, by microarray and sequencing, using peripheral leukocyte mRNA samples from the MRS cohort has identified genes, pathways and networks related to innate-immunity and interferon signaling in association with the development of post-deployment PTSD (Breen et al., 2015; Glatt et al., 2013). Here, we used the causal upstream-regulator-analysis (URA) to predict transcription regulator activity, as well as cytokine and growth factor activity using as input the MRS RNA-sequencing (RNA-seq) data (GEO GSE64813)

from 47 Marines that developed post-deployment PTSD and 47 Marines that did not develop post-deployment PTSD, sampled at pre- and post-deployment time points (1 month prior and 3 months after a 7 month deployment, respectively). The basic principle on which URA is based is that a master regulatory protein can coordinate expression of complex gene networks and that such regulatory proteins can have vastly different roles depending upon its downstream target genes leading to either their upregulation or their downregulation (Kramer et al., 2014). We performed the URA of the pre- and post-deployment differentially expressed gene (DEG) sets identified based on post-deployment PTSD diagnosis (Breen et al., 2015) and predicted differential activity of upstream regulators at pre- and post-deployment based on post-deployment PTSD diagnosis (Supplemental Dataset S1).

From the predicted master regulatory proteins with a significant enrichment of their target genes, **22 transcription regulators** (Fig. 1A) and **31 cytokines/growth factors** (Fig. 1B) displayed a noteworthy absolute value of activation z-score (more than 2) at pre- and/or post-deployment. Already, at pre-deployment transcription regulator activity confirmed a strong innate immune response and interferon signaling (Fig. 1B) operating in the presence of low GR signaling (NR3C1 negative z-score in Fig. 1A) in individuals that will develop post-deployment PTSD, indicating that these mechanisms are potentially vulnerability mechanisms.

Interestingly, at 3-months post-deployment the signal of a portion of the transcription regulators was less strong or in the opposite direction. For the transcription regulators, the largest difference (from pre- to post-deployment) in activation z-score was observed for: **CCR4-NOT transcription complex subunit 7** (↑2.425; pre: de-activation, post: -), **signal transducer and activator of transcription 3** (↓1.912; pre: activation, post: non-significant activation), **breast cancer 1** (↓1.842; pre: activation, post: non-significant activation), **Interferon regulatory factor 1** (↓1.796; pre: activation, post: activation), and **GATA-binding factor 1** (↓1.759; pre: non-significant de-activation, post: de-activation). For the cytokines/growth factors, the largest difference (from pre- to post-deployment) in activation z-score was observed for: **IL-6** (↓4.658; pre: activation, post: non-significant de-activation), **interleukin 4** (↓3.987; pre: non-significant activation, post: de-activation), **Oncostatin M** ↓3.865; pre: activation, post: non-significant activation), **IL-1β** (↓3.769; pre: activation, post: non-significant activation) and **transforming growth factor beta 1** (**TGF-β1**: ↓3.504; pre: non-significant activation, post: de-activation).

The relative changes of these upstream regulators at post-deployment compared to pre-deployment reflect an interaction of pre-deployment vulnerability/resilience with deployment stress effects. For instance, given that the inflammatory IL-6 and IL-1β are reported to be elevated in chronic PTSD populations (Passos et al., 2015), the relative decrease of the group difference of each regulator in post-deployment compared to pre-deployment possibly reflects that both the PTSD and non-PTSD groups elevate transiently the activity of this type of regulators in response to stress narrowing down the group difference at the post-deployment time point, but at the chronic state possibly the difference between the groups becomes large again due to successful adaptation in the non-PTSD group. In contrary, there is no between group difference in the activity of the anti-inflammatory TGF-β1 at post-deployment, but the non-PTSD group has a greater activity

than the PTSD-group at post-deployment. This possibly indicates that TGF-β1 is implicated in stress resilience.

Peripheral mediators of susceptibility in animal models of stress-related disorders

Glucocorticoid signaling and more recently peripheral inflammation has been suggested to be a key factor for the development of stress-related behaviors in animal models (Daskalakis et al., 2013c; Hodes et al., 2015). In this context, an important recent animal study supports that pre-stress individual differences in peripheral immune compartment predict post-stress individual differences in social avoidance behavior (Hodes et al., 2014). They utilized a 10 day repeated social defeat stress (RSDS) paradigm, which induce significant behavioral abnormalities including social avoidance, anhedonia and blunted circadian rhythms in a subset of vulnerable mice (Krishnan et al., 2007). Prior to RSDS, mice that develop the post-RSDS vulnerable phenotype exhibited higher levels of circulating leukocytes than those that were resilient, and did not develop any significant behavioral abnormalities. Higher number of leukocytes prior to RSDS was associated with lower post-RSDS social interaction ratio (time spent in the interaction zone when the conspecific is present by the time spent when the conspecific is absent).

Further examination of specific leukocyte subtypes showed increased levels of pro-inflammatory LyC6hi monocytes (Powell et al., 2013). Moreover, 20 min after the first defeat, some serum cytokines and chemokines were increased in both resilient and vulnerable mice, while others were elevated only in vulnerable mice (IL-1β, IL-6). Both *in vitro* and *in vivo* IL-6 release was higher in vulnerable mice. IL-6 levels showed significant negative correlations with the social interaction, once again indicating a predictive relationship between peripheral inflammation and post-stress vulnerable behavior. These studies confirm that preexisting individual differences in the peripheral immune system of inbred C57BL/6 mice predict post-stress behavior, suggesting that increased post-stress immune activation may be a risk factor for stress-related mental disorders (Hodes et al., 2014).

To examine the functional contribution of circulating leukocytes to stress vulnerability, the authors generated stress-vulnerable bone marrow (BM) chimeric animals by reconstituting peripherally irradiated animals (which removes host immune cells) with donor BM hematopoietic progenitors isolated from vulnerable mice. Control mice received BM from an unstressed donor. After full donor hematopoietic cell reconstitution the percentage of leukocytes derived from donor progenitor cells in both vulnerable and control BM chimeras did not differ; however, the stress-vulnerable BM chimeras had a higher absolute number of circulating leukocytes compared with wild-type control chimeras. More than 95% of the microglia remained of host origin, suggesting that the host microglia population was left largely intact. Vulnerable BM chimeras showed a greater social avoidance behavior compared with control BM chimeras following subthreshold social defeat stress as well as a purely emotional social stress paradigm termed “witness stress”, in which “witness mice” witness other mice being defeated by an aggressor mouse (Hodes et al., 2014).

Given these intriguing results, it will be important for future studies to replicate these results using other models of stress-related individual differences. For instance, it would be interesting to use models that don't require chronic stress exposure and physical injury, which themselves cause chronic inflammation. A single, brief exposure to severe stress, followed by stress recovery, also leads to behavioral abnormalities and brain cellular changes (Cohen et al., 2003; Kohda et al., 2007; Rao et al., 2012). Using the predator-scent-stress (PSS) model, we have recently described the transcriptional signature in blood and brain associated with trauma-related individual differences (Daskalakis et al., 2014). In that study we identified Sprague-Dawley rats with a vulnerable phenotype (high anxiety, fear and arousal), and with a resilient phenotype (low anxiety, fear and arousal) 7 days after PSS exposure (25% each group), while 50% rats displayed an intermediate phenotype. To explore the molecular signature underlying these individual differences, we performed expression profiling in blood and two limbic brain regions (amygdala and hippocampus) in these two extreme groups of animals and in an unexposed control group a day after behavioral phenotyping. The inclusion of controls allowed distinction between genes associated with stress-exposure and those associated with high (vulnerable) or low (resilience) PTSD-like behaviors. There was a small overlap between vulnerability- and resilience-associated genes in all tissue, which supported the concept that stress resilience is a separate biological entity from stress-vulnerability. This suggests that achieving stress-resilience is not a matter of reversing gene signatures associated with vulnerability, but rather, actively engaging resilience genes that decrease behavioral responses to stress. These two distinct entities might operate in a cell-type specific fashion within each tissue (Gafford and Ressler, 2016). Furthermore, amygdala enrichment with vulnerability genes suggested a preferential activity towards the vulnerable phenotype, while such specificity was not observed in hippocampus or blood.

In the hippocampus, chronic stress causes dendritic atrophy, loss of spines, and impairs synaptic plasticity, while in the basolateral amygdala (BLA) chronic stress can strengthen the structural and functional basis of synaptic connectivity through dendritic growth/spinogenesis and enhanced long-term potentiation, respectively (Vyas et al., 2002). It should be, however, noted that not all stress paradigms consistently produce morphological changes as seen in the studies by Vyas el al. (Christoffel et al., 2011b). It is interesting to speculate that this heterogeneity of structural changes may represent differences in resilience and vulnerability. Consistent with this notion, individual differences in the behavioral response to stress were also associated with neuronal morphology changes (Champagne et al., 2008; Mitra et al., 2009; Oomen et al., 2010). Using the PSS-model (Cohen et al., 2014; Zohar et al., 2011), Golgi-Cox staining and Sholl analysis revealed that vulnerable animals displayed lower dendritic complexity (total dendritic length, fewer branches and lower spine density) in hippocampal neurons 8 days after PSS, while resilient animals were indistinguishable from unexposed controls in both brain regions (Fig. 2 - data described and quantified before in: (Cohen et al., 2014; Zohar et al., 2011)). Specifically, lower total dendritic length, fewer branches and lower spine density were observed in the DG granule cells. In the CA1 region, both apical and basilar pyramidal neurons were similarly affected, whereas in the CA3 region specific lower dendritic arborization was observed only apical neurons. Interestingly

in the BLA, there was a remarkable increase in length, number and spine density of pyramidal neurons (Fig. 2).

Because most of DEG (>90%) associated with individual differences in the PSS model were found only in one tissue-type, it was of interest to identify convergent transcription regulation across tissue, which could serve as a basis of brain-relevant blood-based biomarkers. A URA using the DEG-signatures computationally predicted **73 activated/de-activated upstream transcription-factors** (TFs). 43.8% of the predicted TFs in the brain were recapitulated in blood. Nine regulators were conserved in all tissues, including the GR, and IκBα (encoded by *NFKBIA*), an inhibitor of NF-κB. GR-signaling was the top convergent signaling-pathway associated with the response variation and GR-agonist administration shortly before (Cohen et al., 2006) or after (Daskalakis et al., 2014) PSS prevented PTSD-like phenotypes, highlighting a novel therapeutic strategy for PTSD. The NF-κB pathway mediates inflammation in the brain orchestrating transcriptional cascades that lead to changes in neuronal structure and function. Increased hippocampal NF-κB signaling has been associated with vulnerability to PSS (Cohen et al., 2011). Interestingly, heightened activation of the NF-κB signaling pathway in the nucleus accumbens also mediates vulnerability to RSDS through regulation of excitatory synaptic plasticity (Christoffel et al., 2011a; Christoffel et al., 2012).

We extended our URA (Fig. 3A) of the above-mentioned amygdala, hippocampus and blood DEG-signatures associated with individual differences to predict the thirty-eight cytokines and twenty-two growth factors that regulate these genes (Supplemental Datasets S2 & 3, respectively). A brain-blood overlap revealed the blood regulators with functional relevance to brain phenotypes; 78.6% of brain cytokines and 47.6% of brain growth factors were recapitulated in blood, while **8 cytokines** (**IFN-γ, IL-1β, interleukin-3, interleukin-5, interleukin-27, prolactin, TNF-α, tumor necrosis factor ligand superfamily member 11**) and **5 growth factors** (**angiotensinogen, epidermal growth factor, fibroblast growth factor 2, nerve growth factor, TGF-β1**) were convergent across tissue (Fig. 3B, in yellow).

Sensing of immune activity by the CNS

In relation to stress vulnerability, the mechanisms that mediate the actions of peripheral immune cells and molecules on brain function are understudied (Hodes et al., 2015). Potential mechanisms described on the communication between the peripheral and central immune system are relevant (Dantzer et al., 2008; Miller and Raison, 2016; Quan and Banks, 2007). Cytokines may get in the brain by passive diffusion or by binding to specific receptors on endothelial cells where they induce local brain production of inflammatory mediators (Menard et al., 2016). There is also evidence that a variety of leukocytes may enter the brain via the circumventricular organs, the choroid plexus and the brain lymphatic system (Lun et al., 2015). Additionally, in situations of peripheral or central inflammation the permeability of the blood-brain barrier is increased allowing the entry of peripheral immune mediators in the brain (Kebir et al., 2007). Once in the brain, cytokines and leukocytes affect neuronal synaptic plasticity by modifying cell signaling and gene expression (Hodes et al., 2015). Moreover, circulating cytokines may also activate the

microglial stress response in the brain, which may lead to persistent synaptic changes (Hodes et al., 2015; Menard et al., 2016).

Future directions for blood-based biomarkers

An important aspect in the understanding of HPA axis and immune dysregulation in PTSD patients is the disruption of diurnal/circadian rhythmicity of the HPA-axis in PTSD (Hall et al., 2015; Landgraf et al., 2014). Compared with subjects without PTSD, subjects with PTSD showed a greater diurnal range due to a reduced trough in cortisol release, which is partially driven by diurnal changes in GC sensitivity and related to sleep fragmentation (Bremner et al., 2007; de Kloet et al., 2007b; Rohleder et al., 2004; van Liempt et al., 2013; Yehuda et al., 1994; Yehuda et al., 1996). The diurnal variation in cortisol output might be related to the increase in pro-inflammatory biomarkers in PTSD (Baker et al., 2012; Rohleder et al., 2004). Interestingly, the first genome-wide association study (GWAS) in PTSD identified RAR-related orphan receptor alpha (RORα) gene to be associated with risk for the disorder (Logue et al., 2013). RORα is rhythmically expressed and regulates the circadian clock gene encoding Aryl hydrocarbon receptor nuclear translocator-like protein 1 (Hall et al., 2015; Landgraf et al., 2014).

The low glucocorticoid tone and pro-inflammatory state are likely to also moderate central cognitive processes, underlying some of the PTSD symptoms. PTSD has been conceptualized as the conditioning of a conditioned stimulus (CS - environmental cue) with an unconditioned stimulus (US – a highly traumatic event). As a result of this contingency, exposure to the CS (and related cues) can produce PTSD symptoms in the absence of the US. Alterations in fear conditioning, extinction learning, extinction retention and sensitization are likely to be involved in the development and/or maintenance of PTSD (Pitman et al., 2012). Although much is known about how the HPA axis affects these memory processes and the respective neuro-circuit (Roozendaal et al., 2009), additional research needs to be done on the role of inflammatory signaling in the regulation of fear memories (Jones and Thomsen, 2013).

Implications for treatment

Some peripheral GC-markers (PBMC methylation of the *FKBP5* proximal promoter, PBMC GC sensitivity, urinary cortisol) associated with PTSD have been shown to change over time in psychotherapy responders, suggesting that GC markers may be targeted for a therapeutic result (Yehuda et al., 2013; Yehuda et al., 2014b). The results of a recent pilot study suggest that hydrocortisone augmentation of prolonged exposure therapy may result in greater retention in treatment (Yehuda et al., 2015a). Responders to hydrocortisone augmentation had the highest pre-treatment PBMC GC sensitivity that diminished over the course of treatment (Yehuda et al., 2015a). In another randomized, double-blind, cross-over trial of mifepristone, treatment was not significantly associated with PTSD symptom improvement, but associated with improvements in verbal learning mediated by the magnitude of cortisol output change during treatment (Golier et al., 2016).

Selective serotonin reuptake inhibitors have been considered in human and animal studies as therapies that can improve PTSD symptoms by normalizing cytokine levels (Tucker et al., 2004; Wilson et al., 2014). Additionally, monoclonal antibodies can be used to sequester peripheral cytokines. This strategy has been deemed effective in the treatment of inflammatory diseases such as rheumatoid arthritis and is currently under investigation for the treatment of unipolar and bipolar depression. It remains to be seen if they are beneficial only to the patients with high inflammation at pre-treatment, as has been demonstrated in treatment-resistant depression (Miller and Raison, 2016; Raison et al., 2013).

Finally, to the extent that low glucocorticoid tone and high peripheral inflammation represent risk factors, targeted therapeutic approaches could be used prophylactically (primary prevention) or immediately following a traumatic event (secondary prevention) based on the genetic makeup of the individual and early life trauma history.

Conclusion

Clarifying the causal relationship between glucocorticoids and peripheral inflammation and increased PTSD risk will be key for the development of targeted treatments. For this, animal models that focus on the individual differences in the behavioral response to traumatic stress need to be used in translational studies (Daskalakis and Yehuda, 2014a). The goal of future research should be to use blood-based measures to identify individuals at risk for developing stress-related mental disorders, like PTSD for which primary or secondary preventive targeted strategies would be successful.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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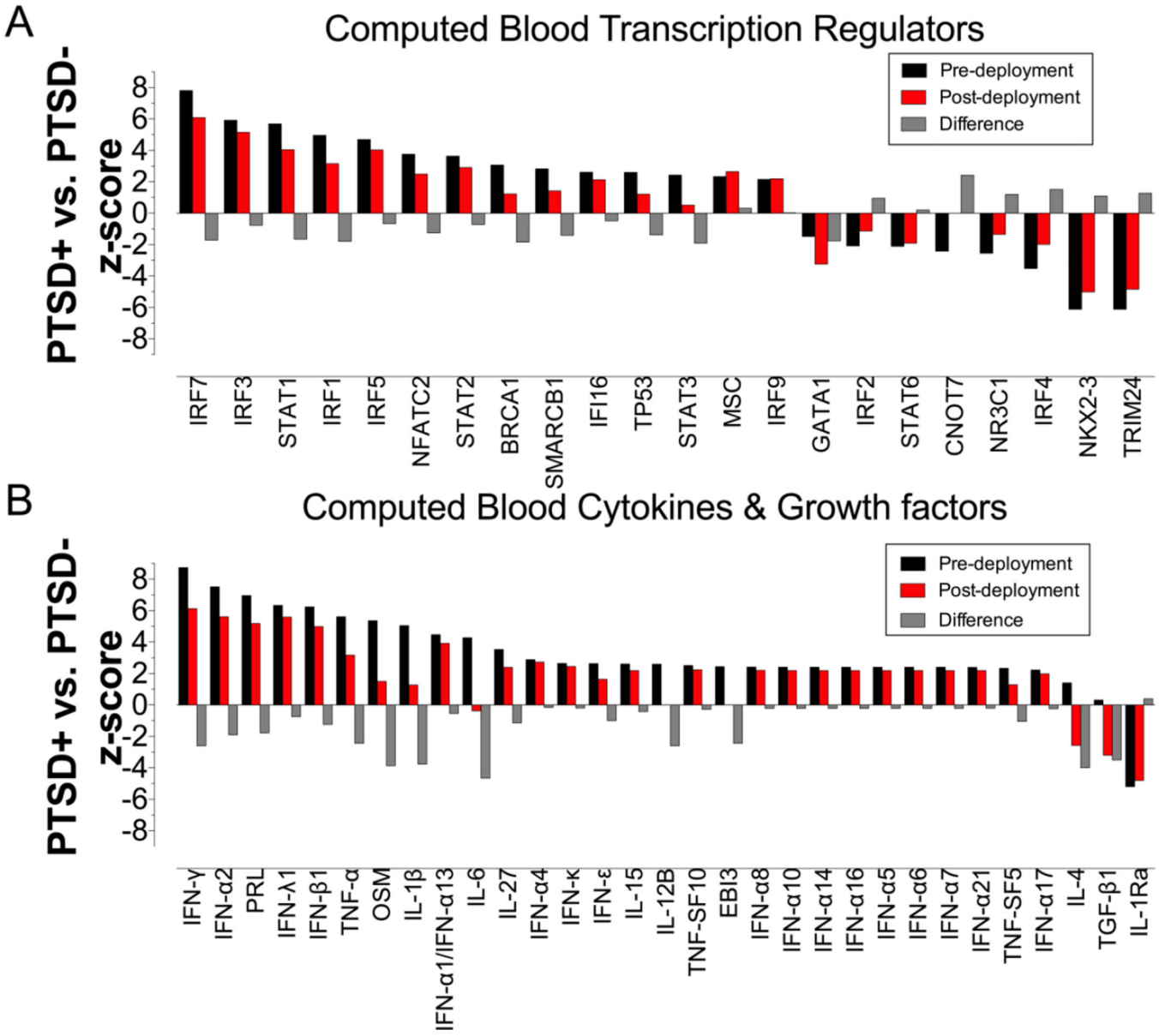

Figure 1. Transcription regulator (A) and cytokine/chemokine/growth factor activity (B) at pre- and post-deployment activity (according to PTSD diagnosis at post-deployment)

Results of gene expression profiling of peripheral leukocytes from Marine Resilience Studies (MRS-II) has been described (Breen et al., 2015). The differentially expressed gene sets at pre- and post-deployment (1 month prior and 3 months after a 7 month deployment, respectively) according to PTSD diagnosis at post-deployment was used to compute the activity of transcription regulators (expressed in a z-score), as well as of cytokines, chemokines and growth factors by upstream regulator analysis (Kramer et al., 2014). The depicted regulators fulfilled the following criteria: (i) significant overlap of target molecules and (ii) absolute value of activation z-score more than 2 at pre- and/or post-deployment (Supplemental Dataset S1). The black bars represent each regulator's z-score at pre-deployment, while the red bars represent the z-score at post-deployment. The grey bars are

the arithmetic difference of the black and red bars. Regulators are ordered from the highest to the lowest pre-deployment z-score.

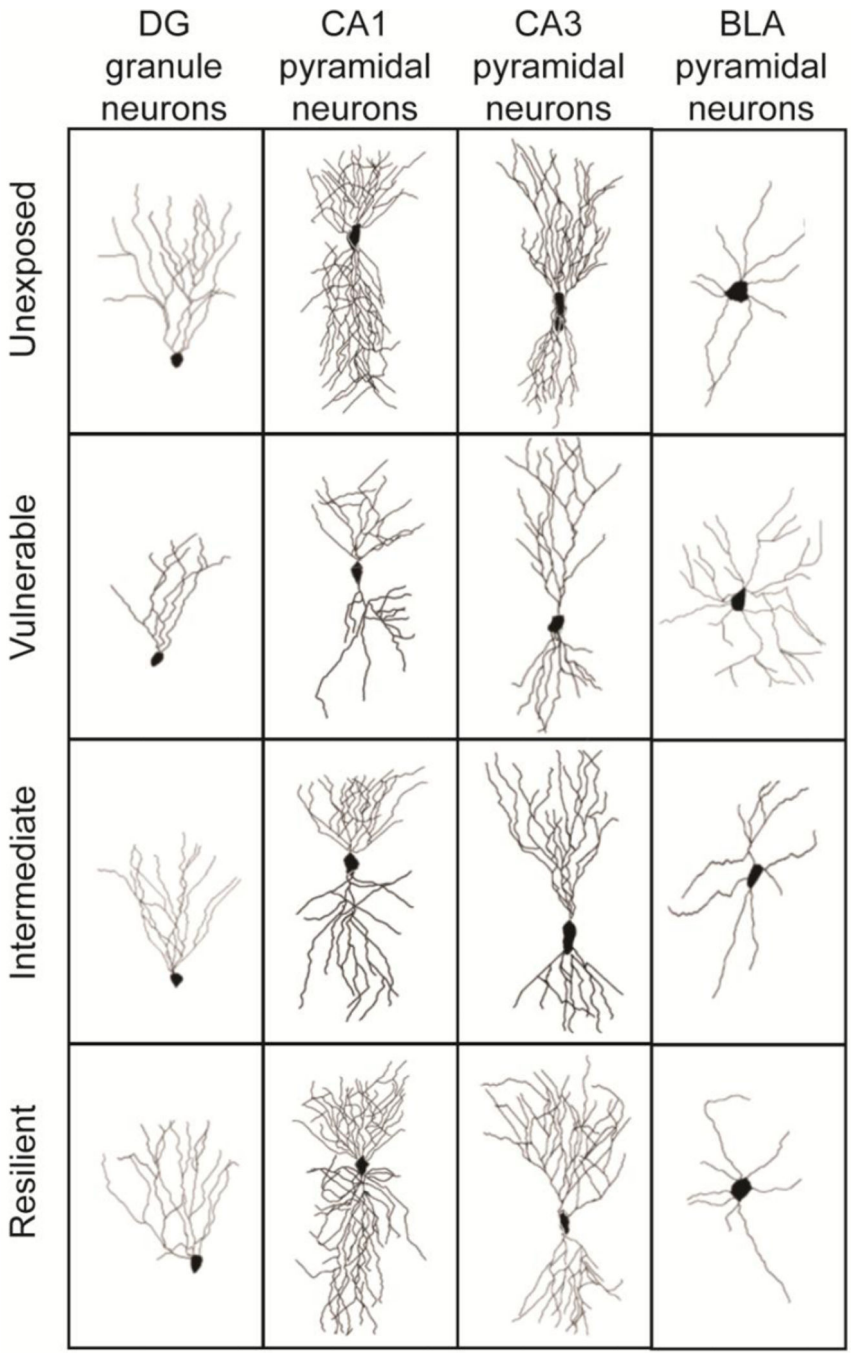

Figure 2.

Computer-generated reconstructions of the dendritic trees of limbic neurons from rats unexposed to predator scent stress (PSS) and rats with a vulnerable, intermediate, and resilient phenotype after PSS-exposure. The depicted data have been described and quantified before (Cohen et al., 2014; Zohar et al., 2011). *DG, dentate gyrus; CA, cornu ammonis; BLA, basolateral amygdala*

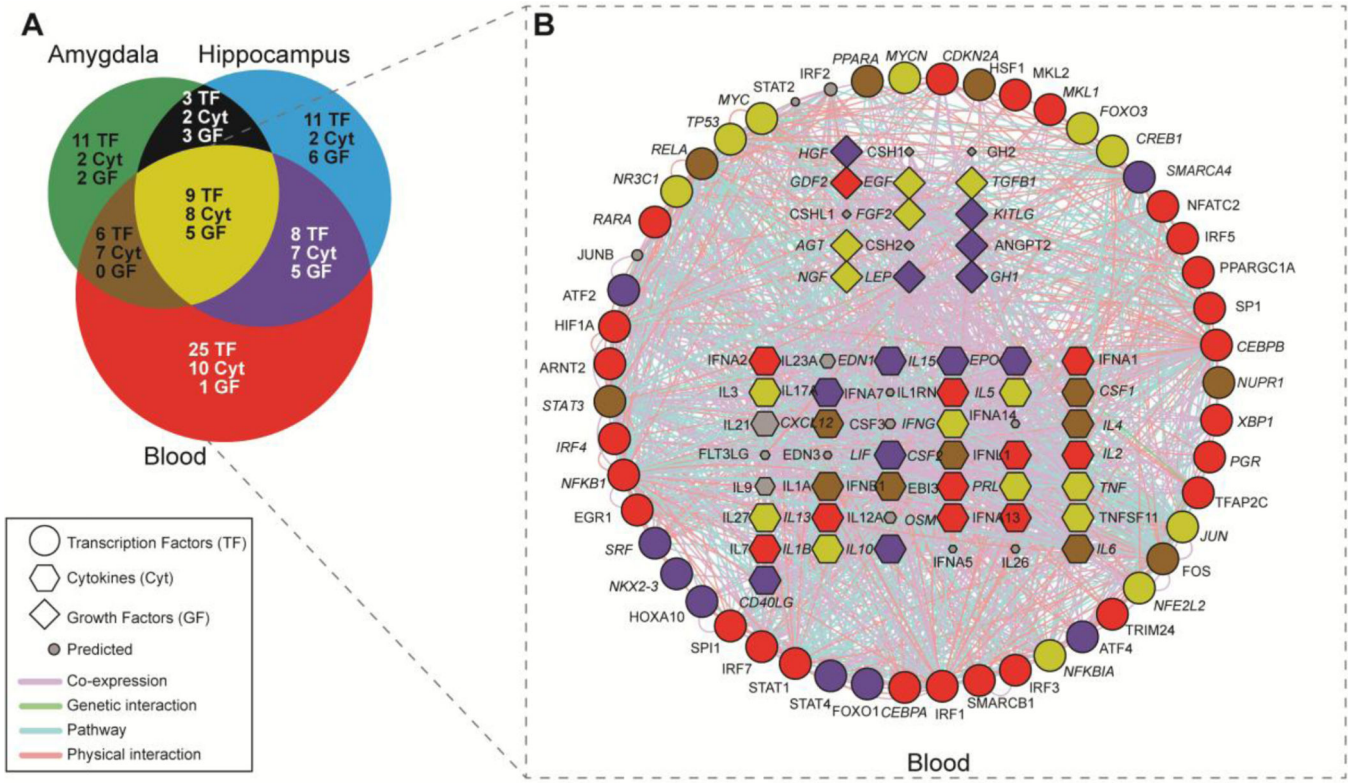

Figure 3. The area proportional Venn-diagram

(a) represents the overlap between the amygdala, hippocampus and blood regulators (transcription factors -TF, cytokines - Cyt and growth factors - GF) predicted by the upstream regulator analysis (Kramer et al., 2014) to regulate differentially-expressed-genes (DEG) associated with individual differences in the behavioral response to predator-scent-stress (described before in: (Daskalakis et al., 2014)). **The gene network of the blood regulators** (b) was built using the GeneMANIA plug-in (http://www.genemania.org/) in Cytoscape (www.cytoscape.org/). The coloring of the network nodes is the same as in the Venn diagram in order to represent the blood-specific regulators (red), the regulators shared between amygdala and blood (brown), between hippocampus and blood (purple), and the convergent across tissue regulators (yellow). The nodes with their name italicized represent that regulate also stress-exposure–associated DEG in the same tissue. The gray nodes are additional interactors predicted by the GeneMANIA gene network analysis; their diameter denotes the prediction score. The between-nodes edges represent relationships and the color of the edges represents the type of the relationship (co-expression, genetic interactions, physical interactions, and common pathway).