Glucocorticoid signaling in a rat model of post-traumatic stress disorder

Ding, J.

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General introduction and outline

Stress disrupts homeostasis

Stress may be defined as the state of the organism in response to a situation that (almost) exceeds our capacity to routinely adapt to it [1]. Diverse stressors activate a wide spectrum of interacting hormonal and neuronal systems to support an appropriate physiological and behavioral response. Behavior refers to the observable motor activities, that are however driven unobservable psychological and neurobiological processes. The behavioral response to stress includes the fight–flight–freeze system (related to fear) and the behavioral inhibition system (e.g., approach–avoidance conflicts, that related to anxiety) [2, 3]. The initial physiological response to stress is mediated in large measure by the neurotransmitter noradrenaline and the hormone adrenaline. The stress response also induces activation of the hypothalamus pituitary adrenal (HPA-axis) which leads to elevated concentrations of glucocorticoid hormones in the blood. These hormones are central to the work in this thesis. In the brain, increased noradrenergic activity, in concert with other mediators such as CRH, is responsible for both physiological and psychological aspects of the stress response [4].

In case of acute and transient stressors, the body's equilibrium quickly returns to normal once the threat is over. A successful acute response to stress is aimed to protect homeostatic balance. However, if the stressor continues over time, chronic stressors may involve a change in homeostatic setpoints, to a less optimal level of functioning, in a process that has been called allostasis [5]. The human body is capable of adapting its physiological processes when faced with repeated or severe stressors. Nevertheless, exposure to such stressors can through increased secretion of stress hormones ultimately result in increased allostatic load (AL) [6]. AL is a measure used to indicate the accumulated strain on physiological responses that surpasses the usual operating limits[7, 8]. This metric serves as an integrated measure of metabolic dysregulation, immune and neuroendocrine in response to stress [9]. AL is thought to cumulatively increase the risk for both physical and mental disease over the life span.

While transient acute stressors are often conceptualized as adaptive, they may contribute to disease if they are very strong. Exposure to such traumatic stressors can lead to (suppressed or overactive) deviant activities of physiological systems, and this can produce sufficient AL to disturb proper tissue- and organ functioning and ultimately lead to a disease state [5]. Post-traumatic stress disorder (PTSD) is the clearest example, and involves not only psychiatric symptoms, but also pervasive physiological impairments [10]. Several physiological disruptions commonly observed in individuals with PTSD have been documented in various systems which are associated with elevated AL [11-15]. The research discovered proof consistent with early or accelerated aging in individuals with PTSD, and the physiological consequences of aging are often linked to elevated AL [16]. However, the acute psychiatric symptoms of PTSD are the main concern in practice, and will be the focus of this thesis.

Stress and PTSD

Feeling scared is a normal response that can occur during and after experiencing traumatic stress. This instinctive “fight-or-flight” reaction is designed to safeguard individuals from potential danger. However, in PTSD the stress-induced changes act on a much longer time scale. PTSD develops only in a subset of people who have experienced an extremely traumatic event. In the most recent version of the DSM-5 (American Psychiatric Association, DSM-5), PTSD is classified into 20 symptoms in four clusters: active avoidance, intrusion, alterations in arousal and reactivity, and negative alterations in cognition and mood. The diagnostic criteria can be summarized as experiencing a stressor and having at least one intrusion symptom in association with it, one avoidance symptom, two negative changes in cognitions and mood-related symptoms, along with two symptoms related to heightened arousal and reactivity, enduring for a minimum period of one month, with functional impairment [17]. The PTSD patients display fear generalization, for example, it demonstrates how hypervigilance and exaggerated reactions towards potential dangers and even irrelevant signals [18]. Clearly military personnel and people with ‘first responder’ occupations (police, firefighter, medics) get regular exposure to various traumatic events frequently and are at high risk for PTSD [19-

21].

Several stress-related signaling molecules may be part of the development of PTSD. Central noradrenalin is related to arousal and vigilance [22]. CRH (corticotropin releasing hormone) is a coordinating factor of the stress response in the brain, which is activated within seconds after exposure to stress and play a central role in the adaptation of the organism to stress [23]. The high levels of glucocorticoid stress hormones secreted by the adrenal gland also may impact on the brain at different levels, and they have been hypothesized to be a major factor toward the development of PTSD [24].

Stress and HPA axis

The increased (nor-adrenalin) signaling upon stress is the consequence of activation of the sympathetic nervous system (SNS), which also includes (indirect) feedback to the brain [25]. The increased levels of glucocorticoid hormones are brought about by activation of the hypothalamic–pituitary–adrenal (HPA) axis [26]. The HPA axis is a slower system. Stress exposure stimulates parvocellular neurons in the hypothalamus produce CRH, which activates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. This in turn stimulates cortisol secretion in humans or corticosterone release in rats from the adrenal cortex (Figure 1A). In response to acute stressors, these glucocorticoids peak at 10 to 15 minutes after the onset of the stress response.

Cortisol is a potent corticosteroid hormone and plays a key role in the body's response to stress. Corticosteroids bind to two receptor types in the brain: the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Disruption of MR and GR signaling is proposed to underlie HPA axis dysregulation seen in stress-related psychiatric disorders [27]. Compared to the GR, corticosteroids have a 10-fold higher MR affinity, and this makes that MR and GR have different roles in the regulation of processes in the brain, including HPA-axis regulation [28, 29]. Its high affinity results in a high MR occupancy even under basal (non-stressful) conditions. This is thought to maintain the excitability of neuronal circuits [30] and helps maintaining low

basal corticosteroid levels through negative feedback on MR in the hippocampus. These effects involve the genomic effects, as MR and GR both act as ligand-dependent transcription factors. In contrast, full GR occupancy is increased when cortisol concentrations peak during the circadian peak or following stress [31]. The genomic effects mediated by GR take effect in the second phase of an acute stress response, typically starting around 30 minutes after the onset of stress. The peak stress concentrations also activate rapid MR- and GR-mediated non-genomic effects, presumably via membrane bound receptors [32]. Negative feedback mediated by GR involves both rapid and slow mechanisms.

The enhancement of memory consolidation for arousing experiences by glucocorticoid hormones is widely recognized [33-35]. Previous work revealed that enhanced corticosterone synthesis during fear learning strengthens the consolidation of fear memory [36, 37]. Effects mediated by GR have been associated with subsequent adaptive mechanisms, like negative feedback systems and the consolidation of recently acquired memories [38]. Corticosterone binding to GR is the principal mechanism for activation of GR to exert its memory-enhance effects [39, 40]. The administration of corticosterone or GR agonist administered into the basolateral amygdala (BLA) or hippocampus has been found to improve memory consolidation in inhibitory avoidance training or in any other training involving a significant contextual component [41, 42]. Of note, recent evidence suggests that while both noradrenalin and glucocorticoids can enhance memory strength, the effect of corticosterone is to also generalize the memories around stressful events [43]. As generalization of memories is highly relevant for PTSD, these findings emphasize the potential of glucocorticoids contribution to the pathogenesis of the disease [44].

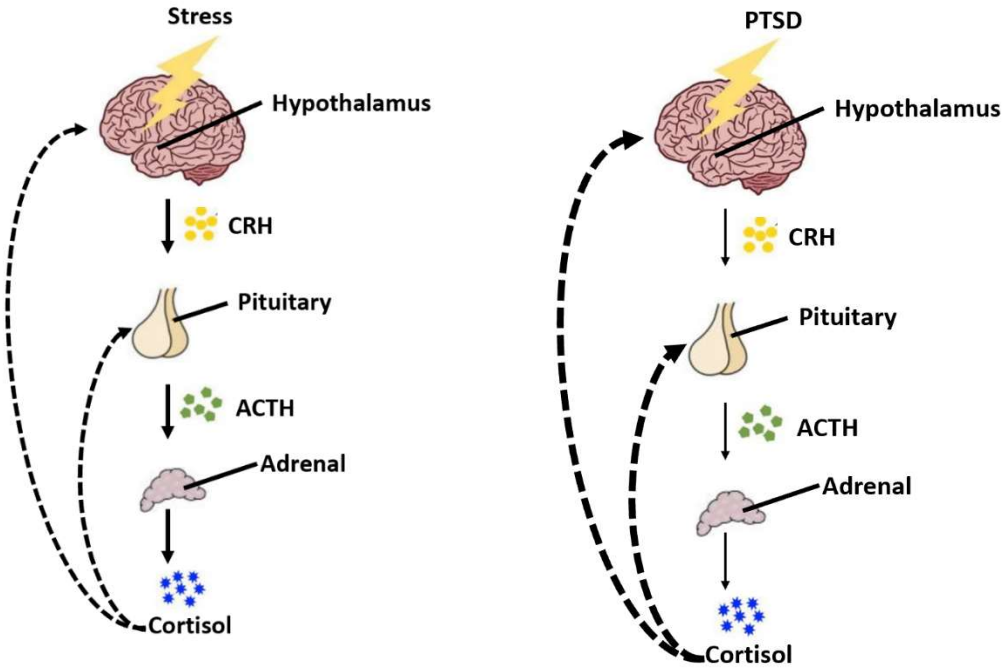

Figure 1. A: Exposure to stress and PTSD results in the release of corticosteroids via the hypothalamic-pituitary-adrenal (HPA)-axis. Cortisol exerts negative feedback on the HPA-axis and prevents a damaging overshoot. B: Most people with PTSD show a low secretion of cortisol and high secretion of CRH in hypothalamus, these suggest that enhanced negative feedback to inhibition of cortisol, itself likely due to an increased sensitivity of GR.

PTSD, the HPA axis: GR sensitivity

The initial implication of GR signaling in the pathogenesis of PTSD was based on the finding that people with PTSD display abnormally low levels of cortisol (and high concentrations of catecholamines) in urine, with (as a consequence) – a higher norepinephrine/cortisol ratio than in comparable healthy individuals [45]. This contrasts the typical acute stressor, in which both catecholamine and cortisol are elevated. With the dexamethasone suppression test, the sensitivity of GR-mediated negative feedback can be assessed. Hypersensitivity of the GR has consistently emerged as a prominent aspect in the impaired functioning of HPA axis in individuals with PTSD [46, 47]. The greater suppression of cortisol following dexamethasone administration demonstrates increased GR sensitivity at the level of the pituitary [48]. It is unknown whether this GR sensitivity generalizes to the brain. In PTSD individuals, increased

GR sensitivity may lead to negative feedback inhibition of cortisol at the pituitary, hypothalamus, or other brain regions comprising - and projecting to - the HPA axis (Figure 1B). Enhanced GR central sensitivity could possibly also be linked to changes in hippocampal volume and potentially impact various physiological systems regulated by glucocorticoids [49].

PTSD and GR genomic target genes

GR gets activated strongly by increases levels of cortisol that follow strong stressors. GR is a member of the nuclear receptor superfamily of ligand-dependent transcription factors. Upon ligand binding, GR translocates to the cell nucleus to enhance or repress transcription of target genes by a diversity of transcriptional mechanisms [50-52]. In the brain, the predominant mode of action seems to involve GR binding specific elements of DNA, termed GREs [53]. Although the genomic action of GRs has been well investigated, which of these actions play a role in the behavioral responses is still not yet very well understood at present. The expression of GR and possibly its downstream targets could serve as potential biomarkers for assessing vulnerability and treatment in (a subgroup of) PTSD patients. The FKBP5 gene is a prominent target gene of the GR. At the same time, FKBP5 protein serves as an inhibitory co-chaperone that prevents GR translocation to the nucleus. Interestingly, genetic variability in the GRE regulating FKBP5 expression was previously linked to vulnerability for negative consequences of childhood trauma – lending further credibility to a role of the GR in PTSD development [54]. Other GR target genes that can be used to assess the strength of glucocorticoid signaling include GILZ and SGK-1, which have been proposed as biomarkers of trauma-related vulnerabilities [55]. In addition, Sgk1 is reported to play a role in cellular and behavioral models of learning and memory [56]. Per1 is a GR-responsive period gene associated with the circadian rhythm, and may play a role in various cellular processes, e.g. the regulation of neuronal function [29].

β-arrestin-2 is glucocorticoid-responsive target gene that is suppressed by GR. This is accomplished at the transcriptional level by the binding of GR to intragenic glucocorticoid response elements (GREs) [57, 58]. β-arrestin-2 is of functional interest as it regulates fear/anxious memory formation, but functions in PTSD are remains unknown.

PTSD animal models

Animal models serve as a vital instrument in scientific research to investigate underlying diseases pathophysiology and neural mechanisms and for the development of novel treatments [59]. The goal of animal research in PTSD include a better comprehension of the intricate interactions among genetic, neuroendocrine and environmental aspects, to identify potential targets for innovative pharmaceutical therapies, and to evaluate drugs for their viability in treating PTSD in humans [60].

In view of the complexity of PTSD, there is no single widely accepted animal model of PTSD, but fear memory abnormalities and HPA axis dysfunction are central features of PTSD patients that should be incorporated in models. At present, numerous stress paradigms in rodents that mimic this behavioral symptom and/or neuroendocrinology alterations in PTSD. For example, fear conditioning (FC) is one of the predominant animal models of PTSD [61]. However, as the formation of fear memory is in principle adaptive, it is mainly the extent to which learned fear generalizes from 'cue' to 'context' or even further that may be considered as an aspect that is relevant to PTSD [43]. Utilizing foot shock models, researchers can effectively replicate several key symptoms of PTSD, including anxiety behavior and avoidance [62], re-experiencing, aggression and hyperarousal [63]. They have revealed that these induce substantial levels of extreme fear and stress in rodents, subsequently leading to enduring behavioral and endocrine stress responses [64, 65]. Other (components of) PTSD models include restraint stress, tail suspension, social isolation, underwater trauma, social defeat, early-life stress, chronic stress, and single-prolonged stress (SPS) [66, 67].

We have used the SPS paradigm in the work described in this thesis. SPS is the first experimental paradigm that could replicate changes in HPA axis similar to those observed in PTSD patients [68]. It reflects the core of PTSD the endocrine phenotype [69], namely negative feedback enhancement. SPS is a protocol that exposes individual rats to three stressors in a sequential and multimodal manner, as a means to mimic traumatic stress. Given that SPS rats mimic both the enhanced glucocorticoid negative feedback and anxiety-like behavior that are

observed in PTSD, the model provides a valuable means to investigate the involvement of the HPA-axis in PTSD [70]. In addition, SPS rats exhibit enhanced consolidation and impaired extinction of conditioned fear memory suggests that this model has additional value [71]. The validity of the SPS model in investigating PTSD are highlighted by its ability to replicate a range of behavioral, molecular, and physiological changes observed in PTSD patients [72].

PTSD and treatment

PTSD is not easy to treat, and treatment options do not suffice to help all patients. It is estimated that approximately 30% of individuals with PTSD do not respond to first-line treatments such as cognitive behavioral therapy (CBT) and antidepressant drugs such as SSRIs [73, 74]. This can be a frustrating and it can lead to a sense of hopelessness and a belief that the condition is untreatable [75]. At present, various therapeutic options have been recommended for patients suffering from PTSD, which mainly include pharmacotherapy and psychotherapy. The latter is often combined with eye movement desensitization and reprocessing (EMDR), many patients have good response to exposure therapy and EMDR. Since the precise mechanisms of PTSD remain unknown, rational (mechanism based) pharmacotherapeutic treatment interventions have not yet been established. The GR has been proposed as a potential factor in the neurobiological processes related to PTSD development or maintenance [76]. The reduced cortisol levels in patients with PTSD have been linked to heightened GR responsiveness or sensitivity, at least at the level of the pituitary [77]. An excessively active central GR may be a crucial factor in the development of PTSD, due to its disruption of adaptive fear memory regulation [78]. In one of the chapters of this thesis we address the question of central GR sensitivity.

If central GR overactivation contributes to the maintenance or development of PTSD, using GR antagonism could be beneficial in preventing the onset of PTSD [79]. Strikingly, the previous studies in rodents has shown that administering the GR antagonist RU486 to adult male rats can restore the negative effects of early life stress. These effects consisted of deficits in contextual memory, altered neuronal activity and increased freezing behavior [80, 81]. The

studies from Papilloud et al. [82] also showed that treatment with RU486 during adulthood successfully reversed the atypical aggressive behavior in rats that experienced stress in prepuberty. In this thesis, we evaluated reversibility of the effects of adult stress by GR antagonist RU486.

THESIS OUTLINE

In this thesis, we investigated the GR sensitivity and behavior in the PTSD. We evaluated the effect of RU486 treatment after rats were exposed to the three consecutive stressors of the SPS model (chapter 2-3). We aimed to identify a sensitization of brain GR signaling that extends beyond direct negative feedback regulation (chapter 4). Lastly, we provide evidence for a role of β-arrestin-2 as a modulator of regulating amygdala activity in response to fear/anxious memory of PTSD (chapter 5).

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