Chapter

Consequences of Chronic Stress on the PINE Network

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Abstract

Stress is a risk factor for the development and progression of a variety of disorders. At the same time stress is essential to initiate adaptation to the current situation and to promote survival of the fittest. Thus, responses to stress evolved to be fast and efficient. This is implemented by a tight networking of the psycho-immune-neuro-energy (PINE) system. Within the PINE network, glucocorticoids are the universal messengers that regulate overall physiology jointly with cytokines, neurotransmitters and energy status. While the secretion of glucocorticoids in response to stress is itself a rather unspecific reaction to any kind of stressor, complexity of the outcome is encoded by lifetime, recent and present events. Together, these individual experiences modulate the diurnal and ultradian rhythmicity of glucocorticoid levels. Given the time- and dose-dependency of glucocorticoid signaling, this rhythmicity allows for flexibility in the coping with stress. In a chronic stress setting, the interaction of PINE network components is altered. While stress-resilient individuals retain adaptive capacity, vulnerable individuals lose flexibility in their responsiveness. Gene × environment interactions could explain individual differences. To better elucidate the molecular underpinnings of risk and resiliency, models that allow studying the consequences of chronic stress on the PINE network are required.

Keywords: plasticity, bioenergetics, inflammation, kynurenine pathway, glucocorticoid rhythmicity, animal model, stress, Fkbp5

1. Introduction

In the past years, the incidence of psychiatric disorders increased. Meanwhile, the majority of absence from work due to illness is attributable to psychiatric disorders. This not only impairs the affected individual but also puts a strong financial pressure on health systems [1]. Commonly, psychiatric disorders are described, classified and treated based on phenotypic symptoms. However, the success of this approach is limited since our understanding of the mechanisms leading to psychiatric pathology is far from complete and explanations to all facets of the disease remain to be discovered [2]. A first starting point to better elucidate the etiology of psychiatric disorders and to offer new treatment options is to better understand the impact of life events on physiology. These environmental influences are known to proceed onset of pathology, and together with some level of genetic susceptibility can alter brain function and overall physiology. Chronic stress is one such environmental factor and is considered a common trigger of psychiatric disorders [3]. Thus, an improved understanding of the phenomenon of stress and its

consequences on physiology will support the discovery of novel treatment options or even preventive strategies. At its core, the chronic or acute inability of an individual to cope with any demand produces stress. This generic definition of stress as a response to unmet requirements proposed by Hans Selye introduces the need of responding to an adverse situation to resolve the stress exerted on the affected individual. The triggers of stress can be internal or external in nature. All non-specific reactions of the body to allow coping with challenges can be summarized under the umbrella term 'stress response'. First, an instantaneous 'fight or flight' reaction mediated by beta-adrenergic signaling introduces a shift from anabolic and restorative processes towards catabolic and energy consuming processes. Secondly, effects of hypothalamic–pituitary–adrenal axis (HPA-axis, used abbreviations are listed in 8) activation come into play to support this potential increase in energy expenditure and coordinate longer-termed stress responses. Glucocorticoids (GCs) are the messengers of this phase of stress response. They are secreted from the adrenal glands to fulfill their eponymous actions on blood glucose levels. In addition, glucocorticoid effects involve the mobilization of fatty acids and amino acids, maintenance of a sufficient blood flow to distribute nutrients and oxygen, the induction of functional changes in mitochondrial dynamics, alertness of the immune system and processing of cues in the central nervous system (CNS). In sum, these actions guarantee the necessary supply of vital tissues with adenosine-tri-phosphate (ATP) to fuel the stress response and to ultimately promote survival. After resolving the stressful situation, the HPA-axis is turned down via a negative feedback loop. Furthermore, alterations in metabolism are reverted and restoration of the emptied energy depots, healing of wounds, and mental processing of the experienced situation takes place. The body returns back to homeostasis, a term coined by Walter Bradford Cannon that translates to 'stability through constancy'. However, if certain stressors occur repeatedly, a change to these default settings might be more cost-efficient. Such a training effect can result in permanent adaptation. This process is termed allostasis, from the greek 'stability through change'. Both, the high flexibility to cope with several stressors and the ability to adapt to them were of evolutionary advantage, since less fit individuals were eliminated. Thus, an efficient and tight networking of systems required for homostasis and allostasis evolved, of which the psycho-immune-neuro-energy (PINE) network is part of.

2. Glucocorticoids are universal messengers in the PINE network

2.1 Glucocorticoids have multiple modes of action

Signaling of the HPA-axis mutually effects the metabolism, CNS, autonomous nervous system and the immune system. This is implemented by pleiotropic actions of glucocorticoids via multiple modes of actions, including non-genomic and genomic components allowing them to exert power on manifold processes. As an example for non-genomic mode of action, intercalation of GCs with plasma and mitochondrial membranes and interaction with membrane-associated receptors has been described, which enables fast-forward reactions [4]. Furthermore, GCs can trigger other non-genomic effects via their target receptors, the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) since these can interfere with cytoplasmic signaling complexes. In the medium-term, the genomic effects of glucocorticoids come into play, which are mediated by both nuclear receptors. Upon ligand binding, they translocate into the nucleus to interact with other transcription factors for example at glucocorticoid response elements (GRE) in the DNA to

transactivate or transrepress a multitude of targets (reviewed in [5]. While GR and MR are ubiquitously expressed, the actual response to GCs varies widely [6].

In light of the high number of genes that is directly or indirectly affected by GR-mediated signaling, this illustrates that a tight regulation of GC signaling is present. At cellular level, this regulation is partly implemented via receptor maturation and

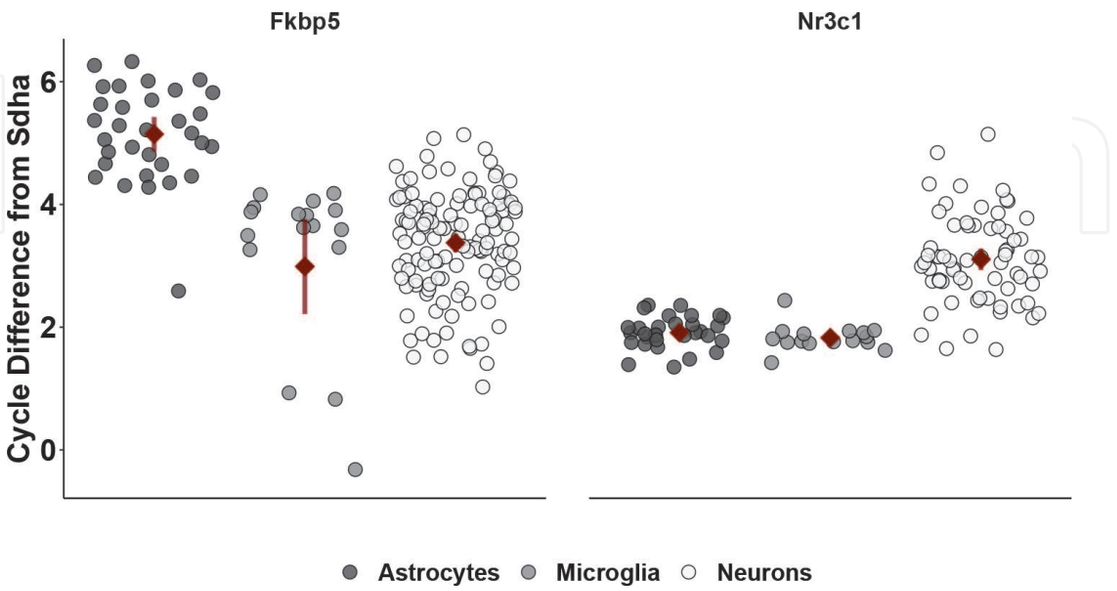

Figure 1.
Experimental details – Primary Cell Culture: *Neuronal cultures were obtained from single cell suspensions of cortex and hippocampus of embryos at E16.5. Cells were seeded in a density of 100,000 cells per 24-well and cultured at 37°C and 5% CO_2 using serum-free neurobasal medium (Invitrogen #12348017) supplemented with Glutamate, SM1 (Stemcell Technologies, Köln, Germany, #05711) and HEPES (Sigma Aldrich, Taufkirchen, Germany, #83264-100ML-F). Conditioned medium exchanges were performed every 3–4 days. On in vitro day 12 neurons were subjected to analyses. Adherent glial flask cultures were derived from enzymatically and mechanically homogenated cortices of neonates. In the culture flasks (75cm² flasks coated with Poly-L-Ornithin Hydrobromid (MW: ≤ 30,000–70,000 Dalton, Sigma #P3655)) the advanced DMEM (Invitrogen #12491015) was supplemented with HEPES, antibiotics and 10% fetal calf serum. Every 3–4 days after 1 week of flask culture, microglia were shaken off and plated at a density of 150,000 cells per 24-well of uncoated PRIMARIA plates (Corning, Germany, #353847) and analyzed in experiments the following day. For plating of astrocytes, flasks after microglia harvest were washed and the astrocyte layer was detached using 0.05% trypsin–EDTA solution (Invitrogen #25300054). Astrocytes were suspended in 50 ml advanced DMEM containing 10% FCS to stop trypsination. This suspension was used to seed the astrocytes into 24-well PRIMARIA plates (1 ml per well). On the next day, a full medium exchange was performed. On post plating day (PPD) 8 the confluent astrocyte layer was treated with AraC-medium (Cytosine Arabinoside, Sigma, #251010, 8μM) for 4 days. On PPD11, the medium was exchanged to LME-medium (L-leucine methyl esters, Sigma, #L1002, 50 mM) for 1 hour and astrocytes were subsequently washed three times with medium. On PPD14, the medium was exchanged to serum-free medium and the assay was performed the next day.* ***Experimental details – qPCR:*** *Native or stimulated cells were lysed in 250μl RLT buffer (Qiagen, Hilden, Germany, #79216) containing 1% beta-mercapto-ethanol (Sigma #M3148-100ML) and frozen at −80°C prior to RNA isolation. RNA was isolated using RNeasy Plus kit (Qiagen #74192) following the manufacturer's recommendations. Integrity was confirmed to be RIN > 8 (Fragmentanalyser, Thermo Fisher Scientific, Langenselbold, Germany). For normalization, 500 ng of total mRNA were used during reverse transcription (high capacity cDNA kit, Qiagen #4368813). All TaqMan gene expression assays were labeled with FAM (Thermo Fisher #4352042; succinate dehydrogenase complex subunit A (Sdha, Mm01352366_m1), murine Fkbp5 (Mm00487403_m1), glucocorticoid receptor (Nr3c1, Mm00433832_m1)) and used in conjunction with the fast universal PCR Master Mix (Thermo Fisher #4351368). Samples were analyzed in technical triplicates on a QuantStudio 6 (Thermo Fisher). All gene expression levels were normalized within the same cell type relative to the cycle thresholds measured for Sdha and for stimulation experiments relative to DMSO-treated cells.* ***Results:*** *Primary murine astrocytes, microglia and neurons differ in their basal mRNA expression of the glucocorticoid receptor (Nr3c1) and its functional inhibitor FK506 binding protein 51 (Fkbp5). Analysis of qPCR cycle number difference from housekeeper (Sdha) revealed that cell types differed in the expression of Fkbp5 ($F(3, 168) = 33.5$; $p < .0001$) which post hoc was attributable to astrocytes displaying the lowest expression of Fkbp5 compared to microglia and neurons. Nr3c1 was higher expressed in both glial cell types compared to neurons (Kruskal-Wallis rank sum test $\chi^2(3) = 64.1$, $p < .0001$). Individual data points are shown alongside with their mean ± 95% confidence interval (red). Shades of gray indicate the cell type: astrocytes (dark gray, left), microglia (gray, middle) and neurons (ecru, right). High values in the PCR cycles needed to reach the set threshold represent low amounts of the targeted mRNA and hence a low expression of the gene while low cycle numbers indicate higher expression.*

turn over. In the cytoplasm, the functioning of the GR is modulated by a molecular hetero-complex that comprises heat shock proteins (HSP), protein phosphatases and a number of co-chaperones. The immunophilin FK506-binding protein 51 FKBP51, encoded by the FKBP5 gene, is one of them. This co-chaperone functionally inhibits glucocorticoid signaling by interfering with the maturation of the glucocorticoid receptor complex. If the GR-HSP90 complex is bound to FKBP51, the

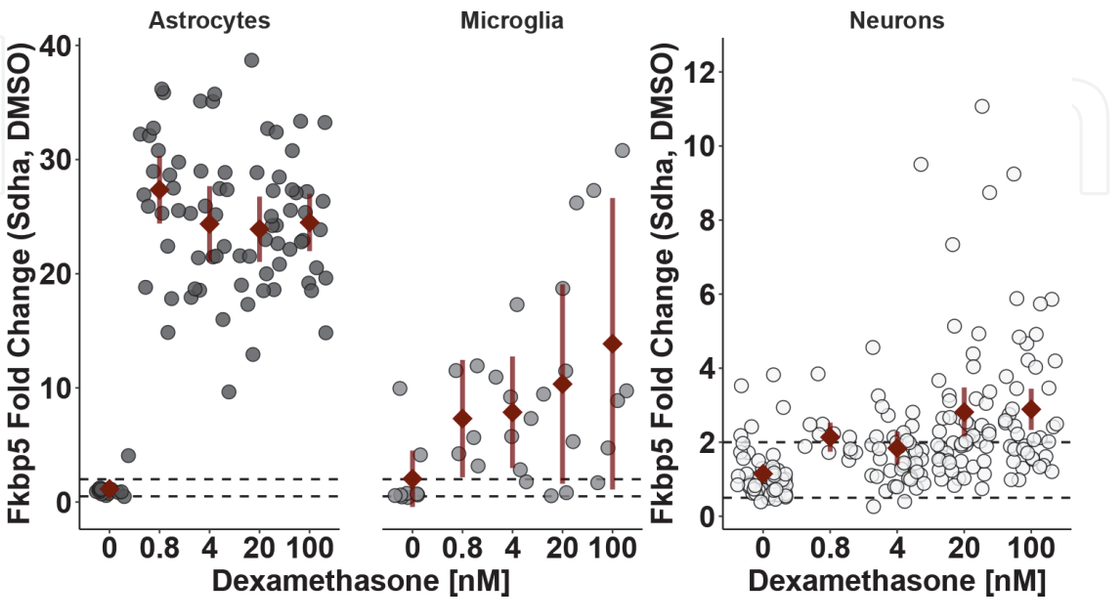

Figure 2.
*Cells were cultured and RNA analyses were performed as described in **Figure 1**. **Experimental details – Glucocorticoid Stimulation:** Stocks of dexamethasone solved in dimethyl sulfoxide (DMSO) were freshly diluted 1:200 in warmed culture medium. Cells were stimulated by replacing 0.5 ml of the medium with the obtained dexamethasone dilutions so that final concentrations of 0.8, 4, 20 or 100 nM were applied. Stimulation was performed between 08:00 a.m. to 10:00 a.m. for 4 hours. To control for manipulation or vehicle effects, a half medium exchange was performed or cells were treated with medium containing 0.005% DMSO, respectively. **Results:** Stimulation with increasing doses of the synthetic glucocorticoid dexamethasone elicited different induction of the glucocorticoid response element harboring Fkbp5 gene, dependent of cell type and dose ($F(8, 309) = 95.9$, $p < .0001$). Astrocytes (dark gray) showed a stronger induction than microglia (gray), while neurons (ecru) were not responsive. Individual data points are shown alongside with their mean ± 95% confidence interval (red).*

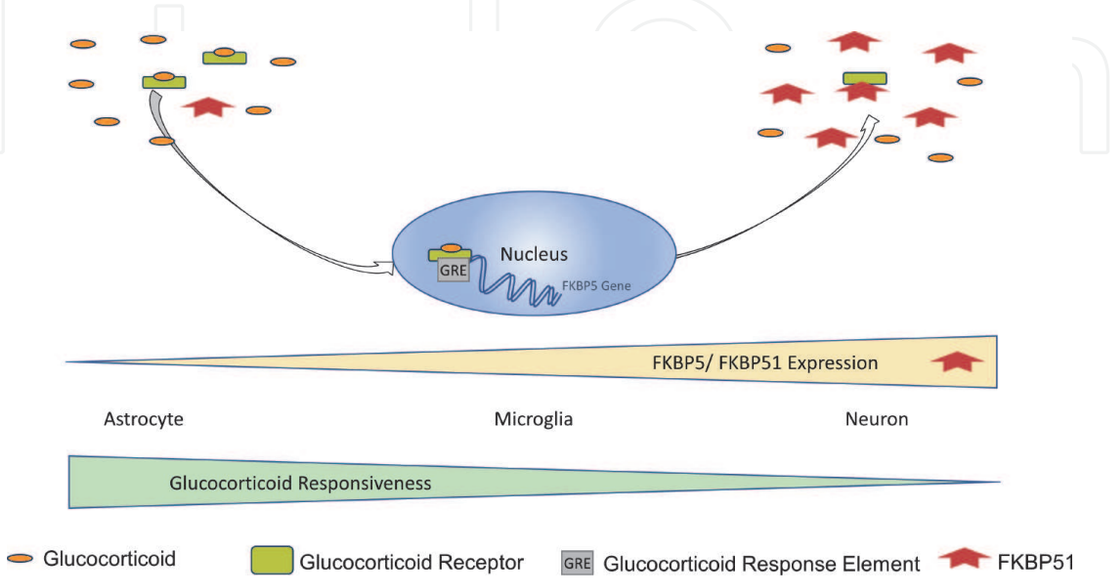

Figure 3.
Mechanistic overview of the interaction between Fkbp5 expression and GC abundancy on GC-induced gene transcription.

GR is in a low affinity state [7]. With these altered dissociation kinetics, more ligand, more GRs or a longer time is needed in order to elicit the same amount of nuclear translocations of the GR as in the presence of fewer FKBP51 molecules. Thus, the abundance of GR and FKBP51 influences the cellular responsivity to GCs and is part of the cellular identity [8]. In the CNS, astrocytes and microglia express the GR at comparable levels, but more than neurons. Astrocytes were found to

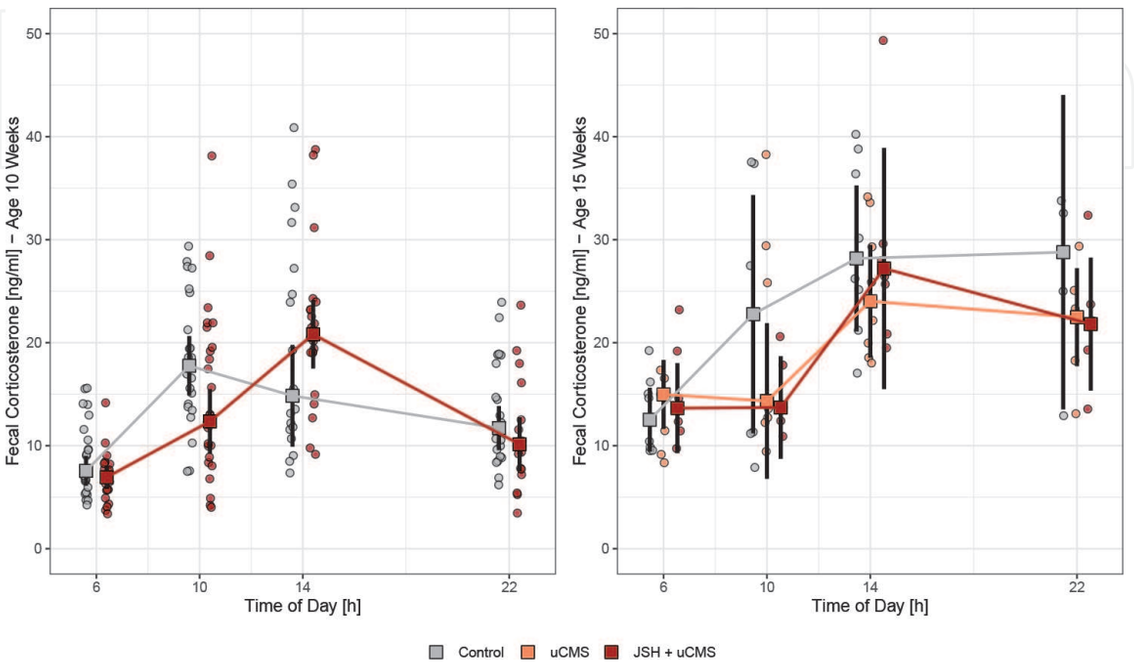

Figure 4.
Experimental details - uCMS: *Test-naïve male Wistar Kyoto rats were obtained from Charles River at an age of 5 weeks and randomly assigned into the experimental groups. All experiments were performed under an inverse 12 hour light cycle (sunrise 18:00, sunset 6:00 with a ramp of 30 minutes). Controls were housed in groups of three throughout the experiment and were sheltered from olfactory, visual or acoustic cues of the stress rats by a Scantainer (Scanbur, Denmark). Rats assigned to the uCMS group were housed in groups of 3 until an age of 10 weeks while uCMS + JSH animals were single housed upon the day of arrival. From an age of 10 weeks onwards, the uCMS protocol started. Only mild stressors such as wet bedding, frequent changes of the bedding, timely limited food and water restriction, intruder confinements, reduction of provided space and flashing lights were applied. Experiments were carried out in male Wistar Kyoto rats under allowance of the regional council for animal welfare (Regierungspräsidium Tübingen, Baden-Württemberg, Germany) and in compliance with directive 2020/63/EU.* ***Experimental details – Corticosterone:*** *Feces samples were collected directly from the animals at 6 a.m., 10 a.m., 2 p.m. and 10 p.m. on the same day and lyophilized. From 50 mg powder of each sample ethanolic extracts were obtained. After evaporation of the ethanol, samples were resuspended in assay buffer and a competitive enzyme-linked immune assay (Cayman Chemicals, Ann Arbor, Michigan, USA) was performed following the manufacturer's instructions. Individual data points are visualized alongside the mean ± the 95% confidence interval.* ***Results:*** *Diurnal Rhythm of Corticosterone Measured in Feces of Rats exposed to Unpredictable Chronic Mild Stress (uCMS) in Young Adulthood with or without Prior Juvenile Single Housing (JSH) in Comparison to Controls.* ***Left:*** *After 5 weeks of JSH the diurnal rhythm of corticosterone was traced in feces of control (gray) and single housed rats (red). Type III sum of square ANOVA of the linear mixed effect model of group-by-time effect corrected for the random effect of each individual animal indicated a significant effect of time ($\chi^2(3) = 92$, $p < .00001$) and group ($\chi^2(1) = 3.9$, $p = .048$). Post hoc tests revealed differences between controls and JSH at 10:00 ($t(169) = 1.97$, $p = .05$). Moreover, in JSH rats a clear peak in corticosterone was observed at 2 p.m. when levels were significantly different from 6 a.m. ($t(135) = 7.3$, $p < .0001$), 10 a.m. ($t(127) = 3.6$, $p < .003$) and 10 p.m. ($t(134) = 5$, $p < .0001$), while between 6 a.m. and 10 a.m. the levels already increased significantly ($t(129) = 4.1$, $p = .0004$). However in controls, the peak was observed earlier at 10 a.m. and levels slowly decreased over noon. This was reflected by significant differences between 6 a.m. and 10 a.m. ($t(130) = 5.1$, $p < .0001$), 2 p.m. ($t(133) = 5.3$, $p < .0001$) as well as 11 p.m. and the 10 a.m. ($t(128) = 2.8$, $p = .03$) and 2 p.m. ($t(137) = 3.1$, $p = .01$).* ***Right:*** *After 5 weeks of uCMS alone (peach) or in addition to JSH (red), the diurnal corticosterone rhythm was traced in feces and revealed a similar pattern for both stress groups that differed from the pattern in controls (gray). While in controls the levels increased over the morning and noon leading to a peak in the afternoon (6 a.m. vs. 2 p.m. $t(53) = 4$, $p = .001$; 10 p.m. vs. 6 a.m. $t(59) = 2.6$, $p = .056$), in the stress groups the levels between 6 a.m. and 10 a.m. remained the same, peaked in the afternoon (uCMS: $t(55) = 2.8$, $p = .03$; uCMS + JSH: $t(55) = 4.1$, $p = .0007$) and began to decrease again towards the night.*

express lower levels of FKBP5 than microglia and neurons, which upon GC-stimulation resulted in a stronger responsiveness of astrocytes, followed by microglia and neurons (**Figures 1–3** modified from [8]). This indicates that abundance of GR relative to FKBP51 imparts a cell-type specific fine tuning of the GC response magnitude at a given time. In addition, this ratio is modulated by recent fluctuations in GC exposure. These modulations are essential for proper functioning [9, 10].

2.2 Origins of dynamic glucocorticoid flows

The exposure of cells to GCs relies on the activity of the HPA-axis. Once triggered, neurosecretory nerve terminals within the hypothalamic paraventricular nucleus are activated to release corticotropin-releasing hormone (CRH) into the portal system of the anterior pituitary, where in response, adreno-cortico-tropic hormone (ACTH) is secreted, transported across the blood–brain-barrier into the peripheral circulation and in the adrenal glands to stimulate the secretion of glucocorticoids into the blood. Over the course of the day, the levels of glucocorticoids undergo substantial fluctuations. While in man cortisol levels peak in the morning, in nocturnal animals like laboratory rodents nadir levels are observed in the morning. Chronic exposure to stress triggers changes in the pattern of this diurnal rhythmicity i.e. shifts in the timing of the peak (**Figure 4** modified from [11]). Besides the diurnal pattern, ultradian rhythms influence the actual plasma levels [12].

These oscillations are enabled via feedback loops between components of the HPA-axis and inside each cell. The feedback occurs at different kinetics and thus introduces phase shifts. Such delays are based on differential glucocorticoid affinity and expression of MR compared to GR, episodic transcription of the rate-limiting enzymes necessary for steroidogenesis, as well as offsets between secretion and distribution of glucocorticoids. In addition, an ultra-short negative feedback loop within each cell is present, since GCs induce the transcription of their functional inhibitor FKBP5 and have the potential to shut down their own signaling [13]. Thus, FKBP5 levels can regulate cellular GC-responsiveness temporally dependent on previous fluctuations in glucocorticoid levels. This generates an additional degree of freedom and flexibility in the stress response system. Interestingly, dynamic changes of GCs are known to be required for normal emotional and cognitive reactions [10, 14]. In humans, several single-nucleotide polymorphisms (SNPs) have been described, which are associated with differential induction of FKBP5 upon glucocorticoid stimulation and thus contribute to the variability of stress perception and coping in the population [15].This illustrates that appropriate negative feedback is required to allow for diurnal and ultradian oscillations of GCs, and that attenuation of the latter goes hand in hand with altered HPA-axis responsiveness and stress coping, which ultimately can impact health. Together, the 24 hours cycle and the ultradian oscillations of GC levels are known to have strong influence on functioning of the body. For reference, the interplay of dynamic GC levels with the PINE network is described in the following sections.

2.3 Biphasic effects of glucocorticoids on the immune system

After their release from the adrenal glands, glucocorticoids are distributed throughout the body via the blood, which is not only the medium for information transportation, but also a home base of the immune system. The reactions of the immune system to glucocorticoids are known to be time-, condition- and dose-dependent. This results in several phases. As part of the fight or flight response, catecholamines are immediately released via the sympathetic-adrenal-medullary

system and trigger the mobilization of monocytes from the bone marrow as a consequence of stress [16]. Glucocorticoids and catecholamines then act together in this preparatory phase with an increased perfusion of peripheral tissues ensuring the energy supply of peripheral tissues for the fight or flight response but also the distribution of the mobilized monocytes. In the event of wounding, blood can flush out pathogens and contribute to an initial sealing of the wound. During the acute phase of stress and high glucocorticoid exposure, the immune system itself is suppressed to reduce inflammation-associated swelling of tissue. Furthermore the liberated energy can be allocated for fighting the current situation rather than pathogens. In the clinic, these immunosuppressive effects of GC are widely exploited in the treatment of inflammatory diseases and autoimmune disorders. On a molecular level, this can be explained by the GC-mediated inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and glucocorticoid receptor signaling which is followed by a downregulation of pro-inflammatory cytokines and modulation of T-cell activity [5]. In contrast to the inhibitory effects of acute, high doses of GCs, in vitro studies suggest that low doses of GCs, which rather elicit mineralocorticoid receptor signal transduction, trigger pro-inflammatory responses in stimulated macrophages [7]. Since exposure to a stressor is potentially linked to subsequent tissue damage and contagion with bacteria or viruses, this mechanism may be implemented to gear up the immune system in preparation for the fighting of potential infections in the aftermath of stress. In addition, glucocorticoids change the expression of cell adhesion molecules on endothelial cells and immune cells. While glucocorticoid receptor signaling in response to high doses of glucocorticoids is associated with decreased expression of adhesion molecules and less extravasation of immune cells [17], mineralocorticoid receptor mediated signaling in response to decreasing GC levels is associated with higher expression of adhesion molecules that facilitates crossing of the vascular wall for immune cells [18]. Outside of the blood vessels, the formerly mobilized monocytes differentiate into tissue macrophages to clear received wounds from pathogens or destroyed cells [19] Taken together, these findings point towards a biphasic mode of action of the GC in the context of immune responses, with the exchange from acute immune suppressive effects of high dose GCs to pro-inflammatory effects as the levels of GCs decrease. Such dynamic responses to stress and glucocorticoids are also observed in other components of the PINE network.

2.4 Wiring of neural stress circuits is shaped by glucocorticoids

The brain is a highly adaptive organ and retains the ability to change throughout life via a process termed (neuro-)plasticity. In response to experiences and learning, plasticity involves the weakening or strengthening of synapses on a cellular level and circuits between brain areas on an anatomical level. Given the individuality of experiences, this results in unique wiring of the brain and could explain why stress has a different meaning for different people under different conditions. During childhood and adolescence the brain is still maturing and undergoes changes that require even more plasticity. During these developmental phases, the processing of inputs is less deterministic than in adults, which on one side enables flexible learning but on the other side puts young individuals at risk to adopt adverse stress coping and emotional processing approaches that ultimately render them more vulnerable to develop psychiatric symptoms [20]. Indeed, stress and trauma have been reported to severely damage the developing brain [21]. Comparisons of normally developed brain functionality, brains from individuals that suffered from early life adversity such as abuse or neglect, or brains of psychiatric patients revealed that a defined set of brain areas is most commonly affected by stress,

namely the hippocampus, amygdala, and prefrontal cortex (PFC). These brain regions are strongly connected and their networking determines what is perceived as threat and how individuals cope with stress and adversity. Protective factors associated with adequate coping include the ability to stay optimistic, a controlled regulation of emotions, high levels of attention set shifting to focus on different aspects of the current situation, the capacity to reflect on experiences and own reactions and higher cognitive abilities required for executive functions. All of these functions are biologically linked within the network comprising the hippocampus, amygdala, and PFC. Upon perception, the medial PFC filters and processes sensory inputs to initiate thoughts and actions in accordance with internal goals, based on previously learned behaviors retrieved from the hippocampus. To orchestrate defensive physiological and behavioral responses, the PFC is connected to the amygdala, the emotion regulation area of the brain, which in turn contributes to sympathetic and HPA-axis activation and intensifies long-term memory consolidation of adverse emotional events in the hippocampus. By dampening emotions produced in the amygdala, the PFC supports maintenance of cognitive flexibility in challenging situations. This indirectly influences learning processes in the hippocampus, but the PFC can also directly dampen hippocampal signaling and thus modulate memory formation. In the context of memory formation, an inverted U-shaped association of glucocorticoid levels and plasticity has been observed. Since the modulation of cellular activity via glucocorticoids was reported to be brain region-dependent, this could indicate that differential expression of GC-responsive receptors play a role in this biphasic pattern [9]. Indeed, activation of GRs in the presence of high glucocorticoid concentrations were reported to impair long-term potentiation (LTP) by high-frequency stimulation and enhanced long-term depression. While low levels of GCs selectively activate MR signaling, which increases LTP via θ-burst stimulation and increased expression of N-methyl-d-aspartate (NMDA) receptors and thus glutamatergic, excitatory signaling. Whether potentially newly formed synapses persist to change memory and ultimately the way how future stressful events are dealt with, depends on their stability. The expression of cell adhesion molecules contributes to the synaptic stability. Like in endothelial and immune cells, glucocorticoids were found to influence the expression of glycoprotein cell-adhesion molecules in [22]. Not only their presence, but fluctuations in the levels of glucocorticoids were reported to be required for plasticity [23], which could add additional explanation why stress-associated fluctuations in GC levels have a strong influence on memory formation. Summed up, the communication of a challenging situation via glucocorticoids is handled in multiple phases, which allows for additional set screws like dose, timing and previous events to fine-tune stress responses in the brain. In the long run, changes in glucocorticoid levels and differential expression of their receptors can influence emotional, executive and cognitive responses to stress by interacting with plasticity and networking of the amygdala, PFC and hippocampus. Of note, this remodeling in response to challenges allows for adaptation to the current environment but also to adequately regulate the assessment of future challenges. Thus, a high level of plasticity is beneficial to continuously update the connectivity of these sensory-defense circuits. Dependent on individual resiliency factors and in particular contexts, stress challenges may result in personal growth regarding the balancing of anxiety, mood control, memory, and decision making. At the same time, frequent stress challenges can strengthen certain connections within the brain. This training effect might be cost-effective in steady environments, but could be maladaptive in case of persistence and lack of reversibility due to reduced plasticity. Optimizing the choice between homeostatic and allostatic processes is a complex task of the PINE network that involves consideration of associated energetic costs.

2.5 Glucocorticoids interact with mitochondria to regulate energy

Mitochondria are the main providers of energy, namely ATP. Besides glycolysis and fatty acid oxidation, the majority of ATP is produced during oxidative phosphorylation. The motor for the production of ATP is an inward rectifying proton gradient across the inner mitochondrial membrane. In the process of generating this gradient, a series of redox-reactions occurs at complex I to V of the electron transport chain (ETC), which consumes oxygen and substrates generated in the tricarboxic acid cycle [24]. According to the endosymbiont theory, mitochondria are

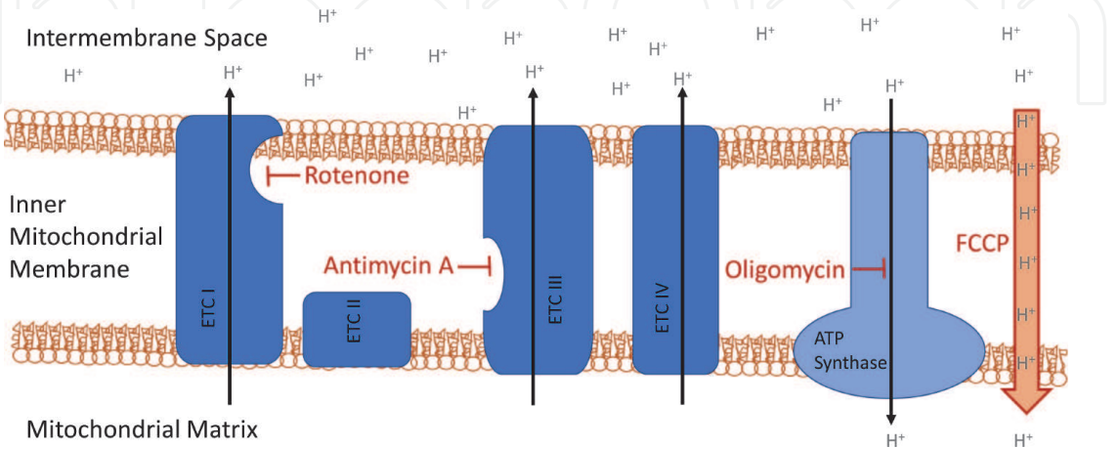

Figure 5.
Schematic of the mitochondrial electron transport chain (ETC) and the sites of action of the inhibitors and uncouplers used to study respirometric performance.

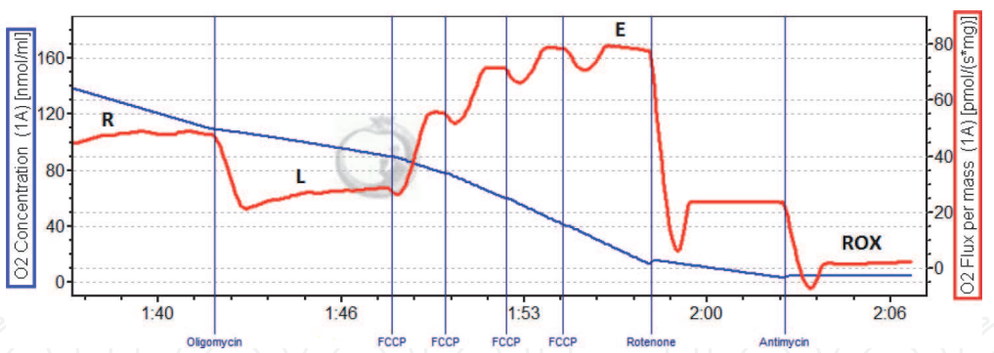

Figure 6.
Animal housing and stress protocols are described in ***Figure 4****. **Experimental details – High Resolution Respirometry:** Rats were sacrificed between 7 a.m. to 11 a.m. under deep anesthesia (intra peritoneal injection of 100 mg pentobarbital/kg body weight, Narcoren®, Boehringer Ingelheim Pharma GmbH & Co KG, Germany). The rostral halves of the right hippocampi were isolated and stored in ice-cold custodial® (DR. FRANZ KÖHLER CHEMIE GMBH, Bensheim, Germany). Immediately before respirometry, the tissue was homogenated, diluted to a concentration of 2 mg/ml with mitochondrial respiration medium MiRO5 (Oroboros Instruments, Innsbruck, Austria) and loaded into the calibrated oxygraph chambers which were pre-warmed to 37°C. Measurements were performed in duplicates. In brief, pyruvate (5 mM), glutamate (10 mM), malate (0.5 mM), cytochrome c (10 μM), ADP (5 mM) and succinate (10 mM) were added to measure routine respiration. By injecting oligomycin (2.5 μM), the ATP-sythase was inhibited and the LEAK state of respiration was induced. Next the uncoupler FCCP was titrated in steps of 0.5 μM to determine the maximal capacity of the ETC. By adding rotenone (0.5 μM), the amount of oxygen consumed independent of complex I was determined. Lastly, antimycin A (2.5 μM) as inhibitor of complex III was injected to measure the residual oxygen consumption (ROX) outside of mitochondria. All chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Company Ltd., St. Louis, Missouri, USA).* ***Results:*** *Exemplary recording of high-resolution respirometry analysis in rat hippocampal homogenate. The blue line represents the oxygen concentration measured via polarographic oxgen sensors over the course of the experiment shown in hours, while the red line indicates its first derivative, the oxygen flux. The injection of inhibitors and the titration of uncouplers of the ETC is indicated by horizontal lines and marks switches between respirometric states: R = routine, L = LEAK, E = maximal electron transfer (in the uncoupled state), ROX = residual oxygen consumption.*

remnants of bacteria which were incorporated as cell organelles into eukaryotic cells. As such, mitochondria still harbor 37 genes on their own mitochondrial DNA (mtDNA), while genes encoding other mitochondrial components were transferred to the nuclear DNA. Glucocorticoids hence can not only influence mitochondria by intercalating into their membranes or by regulating the expression of nuclear genes relevant for mitochondrial function, but also directly interfere with mtDNA in a time and dose-dependent manner [25]. These interactions guarantee a sufficient energy supply during stress. In a chronic mild stress study carried out in Wistar Kyoto rats, an adaptive activation of the ETC and higher respirometric performance was observed (**Figures 5**–**7**, modified from [11]). However, increased activity of the ETC leads to the production of reactive oxygen species (ROS). Complex I activity is associated with more production of ROS than complex II [26]. In a defined manner, ROS serve as important signaling molecules [27] and are essential for the oxidative burst observed in granulocytes to fight microbial infections. In higher doses, ROS can outbalance anti-oxidative defense system which results in oxidative stress [28]. In that event, proteins, lipids and DNA becomes damaged and lose functionality. Based on their microscopic structure and cellular location, mitochondria are especially vulnerable to oxidative stress [29]. In the long run, chronically elevated mitochondrial activity can thus result in a decompensation of the energy providing system. A shift away from ETC complex I towards complex II, as seen in the above cited chronic mild stress study, might be a possibility to reduce ROS overload and to evade the risk of oxidative stress. In addition to their bioenergetic role,

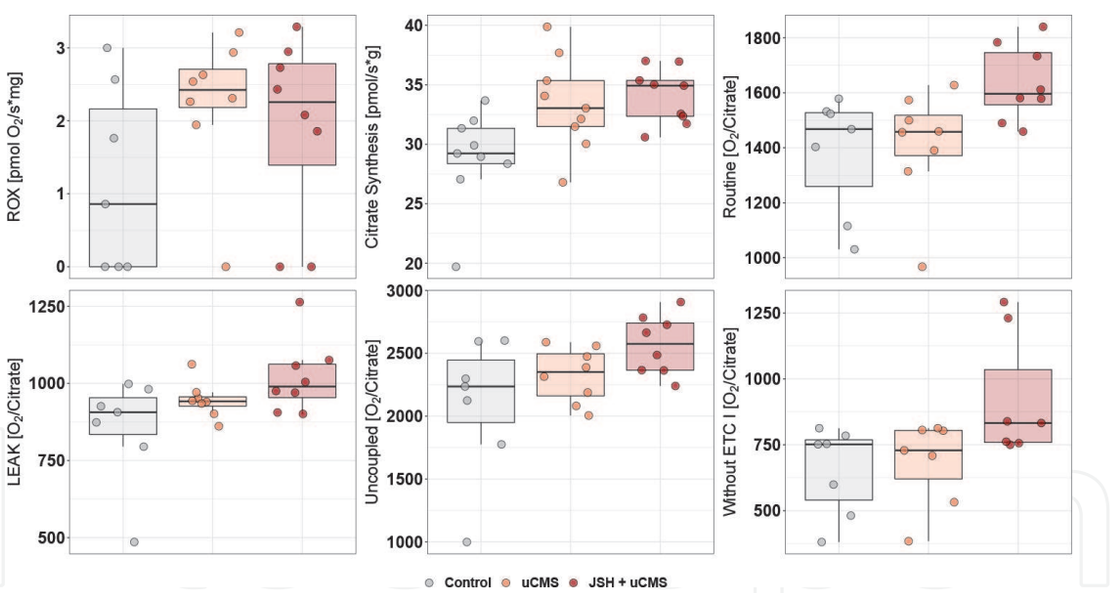

Figure 7.
Animal housing and stress protocols are described in ***Figure 4*** *while the respirometry protocol is illustrated in* ***Figure 5****. **Experimental details – Citrate Synthase Activity:** By coupling the synthesis of citrate and CoA-SH from oxalacetate and acetyl-CoA with the formation of TNB out of DTNB and CoA-SH, the activity of the citrate synthase in frozen homogenates of hippocampus samples was measured spectro-photometrically as the rate of increase in absorbance. In brief, 0.1 M triethanolamine HCl buffer, oxalacetate (10 mM), DTNB (1.01 mM) and the citrate synthase standard were freshly prepared on every experimental day. Distilled water was loaded into 1 ml glass cuvettes together with 100 µl of DTNB, 50 µl oxalacetate, 25 µl acetyl CoA and 25 µl Triton and sample or standard. The absorbance was measured at 32°C.* ***Results:*** *Comparison of the respirometric performance of permeabilized hippocampal homogenate derived from 16 weeks old controls (gray), rats that underwent 5 weeks of uCMS during young adulthood (peach) and rats that were subjected to 5 weeks of JSH prior to uCMS (red). In both stress groups, an increase in mitochondrial mass, measured via citrate synthase activity, was detected ($F(2,24) = 5.7$, $p = .009$; uCMS: $t = 2.7$, $p = .03$; JSH + uCMS: $t = 3.1$, $p = .01$). After normalization to this enzyme activity, statistically significant effects of stress on routine ($F(2,20) = 5.39$, $p = .03$) and respiration without ETC complex I ($F(2,18) = 3.7$, $p = .03$) was suggestive. Tukey's honest significant difference post-hoc testing confirmed that in the double-stress group (JSH + uCMS) compared to controls the routine respiration ($t = 2.6$, $p = .04$) and respiration without ETC complex I ($t = 2.7$, $p = .04$) was increased.*

mitochondria are involved in regulation of apoptosis and calcium homeostasis which were shown to be modulated by GC signaling [30, 31]. This contributes to their key role in regulating synaptic transmission, brain function, and cognition [32]. Taken together, mitochondria are an interesting platform for further communication of GC signaling [33]. Notably, the communication from the HPA-axis to mitochondria is not unilateral, because mitochondria are the site of glucocorticoid production. As such, they express stress-inducible translocator proteins (TSPOs) that modulate oxidative stress and transport cholesterol from the outer to the inner mitochondrial membrane [34]. In addition, mitochondria harbor enzymes required for the cleavage of nutrition-derived cholesterol into precursors of GCs as well as enzymes involved in the conversion of the inactive 11-deoxycortisol or deoxicorticosterone to the bio active cortisol and corticosterone. Thus, mitochondria regulate GC availability and are an additional set screw in the complex feedback structure of GC signaling and the stress response system.

3. The brain recaptures networking of PINE components

Given the importance of the PINE network for stress responses and health, additional ways of communication between its components in addition to GC signaling evolved. The brain is a central hub for the orchestration of stress responses. At the same time it is anatomically rather isolated from the rest of the body and thus contains highly specialized cells that generate functional output and cells that support, shape and surveil the activity in that micro model of the body. The following sections issue in more detail how these tasks are shared in the central nervous system and how chronic stress modulates this.

3.1 Microglia govern the immunity of the brain

In the brain, full blown immune reactions including sudden tissue loss would be deleterious for the fine-tuned neuronal circuits and networks. Therefore, the brain is especially protected from wounding via the skull and a tight interface of astrocytes, pericytes and endothelial cells, termed blood brain barrier, limits the access of blood-born immune responses to the brain. As replacement for the peripheral immune cells, the brain harbors specialized tissue-resident immune competent cells, the microglia. These belong to the monocyto-phagocyting-system like macrophages and are of mesenchymal origin. Besides their phagocytic properties to clear debris, microglia contribute to the pruning of synapses during development and learning. In addition, microglia have a ramified shape in the resting state and monitor the brain parenchyma for pathogen associated molecular patterns or danger associated molecular patterns. Upon detection of such patterns, microglia become activated and change towards a more amoeboid shape that allows for increased mobility. In parallel, different receptors like the cannabinoid receptor 2 or toll-like receptors become expressed on their surface to guide microglia via chemotactic signaling to the site where the activating signal originated from. Once activated, microglia proliferate and produce inflammatory cytokines like interleukin 1 β, interleukin 6 or tumor necrosis factor α. Analogous to inflammation in the periphery, these stimuli trigger clonal expansion and attract more immune cells. Normally, all immune cells in the brain are derived from a residual microglia pool, but in case of severe inflammation the blood–brain-barrier becomes leaky and other immune cells can enter the brain. The latter implies a certain neurotoxic effect of proinflammatory responses initiated by microglia. To prevent this take over from peripheral immune cells and the associated risk for loss of functional connectivity in the brain, the brain

modifies behavior of an individual with potential dangerous infection, as measured by elevated pro-inflammatory cytokine levels, in such a way that the individual withdraws from demanding activities to allow allocation of more energy to fighting the source of infection. This phenomenon is termed 'cytokine induced sickness behavior' and besides altered neurotransmitter signaling, GCs are mediators of this switch that shall protect the brain from severe inflammation. Theoretically, it should also prevent the infected person from entering the general population and spreading the disease. After resolution of the inflammatory insult, returning to a normal state is essential for brain physiology. This involves apoptosis of invaded peripheral immune cells, a switchback to resting microglia or apoptosis of activated microglia as well as tightening of the blood brain barrier.

3.2 Astrocytes provide (metabolic) support to neurons

Astrocytes are an essential component of the blood brain barrier and thus play a crucial role in the protection of the CNS from peripheral cues. In addition, astrocytes regulate the flow of nutrients. This enables astrocytes to metabolically support neurons [35]. For example, astrocytes are involved in the glutamate-glutamine cycle and catabolize glucose via the tri-carboxic acid cycle, which generates lactate that is shuttled to neurons to allow them to directly perform oxidative phosphorylation. While glycolysis produces only 2 ATP molecules from one molecule glucose, oxidative phosphorylation is more efficient and produces between 30 and 36 ATP molecules, dependent on proton leakage across the mitochondrial membrane [36]. In the presence of GCs, this alternative energy source for neurons becomes especially relevant, since GCs reduce the cellular uptake of glucose and glutamate [37]. Enhanced clearance of the synaptic cleft from glutamate by astrocytes could therefore be another way to safe-guard neurons from short-comings in energy. Besides their supportive role in terms of metabolism, astrocytes were shown to influence information processing and cognition by integrating local sensory information and behavioral state [38, 39]. In response to glucocorticoids, astrocytes were reported to directly influence (emotional) learning by regulating neurogenesis and structurally reorganizing neuronal networks [40]. This is possibly due to their role in stabilizing synapses and their responsibility for the rapid clearance of the synaptic cleft. As an example, astrocytes express excitatory amino acid transporters (EAAT1–5) to remove glutamate from the synapse and furthermore recycle it for further use in neurons [41]. Astrocytes hence play an important role in shaping plasticity in response to emotional stress and set the stage for future stressful encounters [42]. In addition, glia cells can regulate neurotransmission by generating neuroactive substances. The kynurenine pathway is one example where balancing of astrocytes and microglia activity is required for adequate modulation of neuronal communication.

3.3 Interplay of CNS-cell types in the kynurenine pathway

The clear distribution of roles between neurons, microglia and astrocytes requires several sites of interaction in order to balance the different activities in the CNS. An example of these interaction points is the kynurenine pathway. The essential amino acid tryptophan is mainly catabolized via this pathway, while only a minor amount (~5%) is used up for the production of the neurotransmitter serotonin. As first step of the pathway, stress-induced GCs lead to an upregulation of the enzyme tryptophan-di-oxygenase (TDO), which converts tryptophan to kynurenine. This enzyme is mostly expressed in the liver [43]. In the brain, microglia can convert tryptophan to kynurenine via the indole-amine-di-oxygenase (IDO) enzyme, which is inducible by pro-inflammatory cytokines. Alternatively,

astrocytes can shuttle peripheral kynurenine across the blood brain barrier into the CNS. From kynurenine, two neuroactive substances are produced, kynurenic acid and quinolinic acid. Under a pro-inflammatory state, microglia dominate kynurenine metabolism and process it via kynurenine-mono-oxygenase (KMO) to quinolinic acid, since KMO becomes highly expressed in the presence of pro-inflammatory cytokines. Quinolinic acid is an NMDA receptor agonist with pro-oxidative capacities. In an anti-inflammatory state, kynurenine may be processed by the astrocytic kynurenine-amino-transferases (KATs) to kynurenic acid. In contrast to quinolinic acid, kynurenic acid has anti-oxidant properties and is an NMDA receptor antagonist. Furthermore, kynurenic acid reduces the release of dopamine and glutamate [44, 45]. An astrocytic dominance of the kynurenine pathway and thus a shift to higher kynurenic acid relative to quinolinic acid thus is considered neuroprotective. However, reduced excitatory neurotransmission could result in reduced synaptic plasticity [46]. In a chronic mild stress study in rats, reduced expression of immediate early genes in the PFC, which suggests a decreased ability

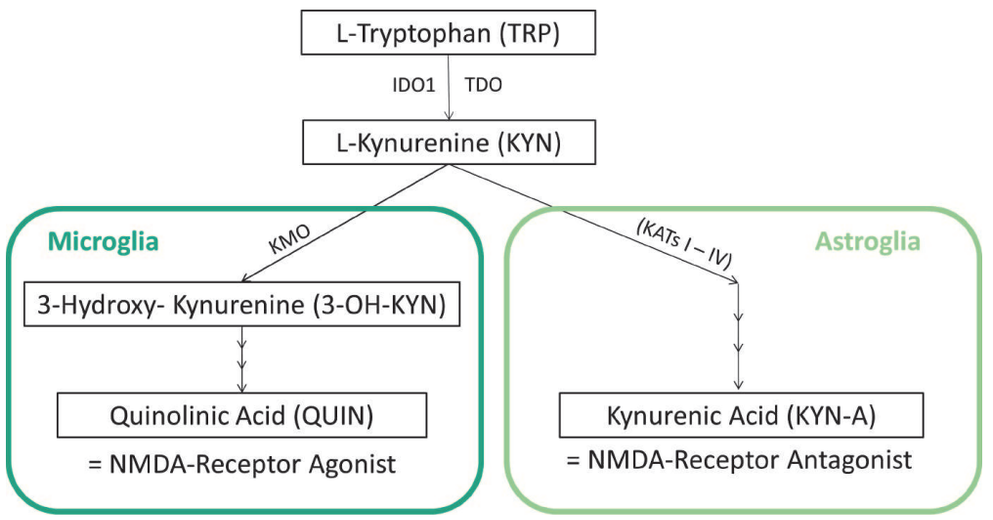

Figure 8.
Overview of trypotophan catabolism by enzymes of the kynurenine pathway in the CNS.

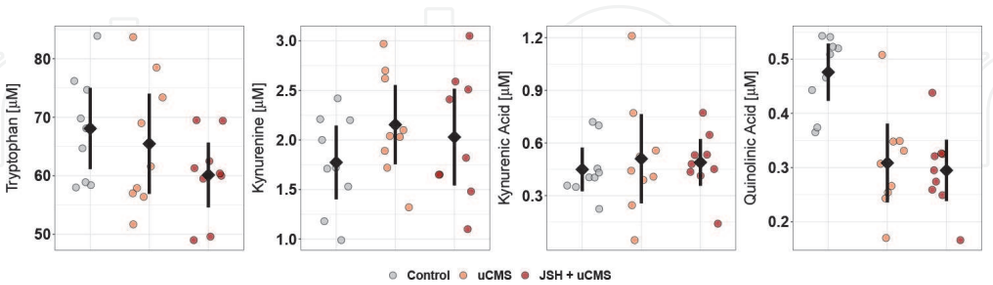

Figure 9.
Animal housing and stress protocols are described in ***Figure 4****.* ***Experimental details – HPLC-MS/MS Quantification of TRYCATs:*** *Tandem mass spectrometry (HPLC-MS/MS) was used for simultaneous quantification of tryptophan (TRP) and its catabolites kynurenic acid (KYNA), kynurenine (KYN) and quinolinic acid (QUIN) in plasma. After protein precipitation with ice-cold methanol, a reversed phase chromatography was performed followed by mass spectrometric detection in the positive ion multiple reaction monitoring mode. Deuterated analogues of the analytes, namely d5-kynurenic acid, d4-kynurenine, and d3-quinolinic acid were used as internal standards. The lower limits of quantification were 625 nM for TRP, 62.5 nM for KYN, 12.5 nM for KYNA and 25 nM for QUIN.* ***Results:*** *Activation of the kynurenine pathway in response to stress exposure. Comparison is shown between controls (gray) and rats exposed to 5 weeks of unpredictable chronic mild stress in adutlhood (peach) as well as rats subjected to 5 weeks of juvenile single housing (JSH) prior to uCMS (red). Data point represent individual animals and are shown alongside with the mean ± 95% confidence interval. In both stress groups, the levels of the NMDA receptor agonist quinolinic acid were significantly decreased compared to controls (F(2,24) = 14.4, p = .0001).*

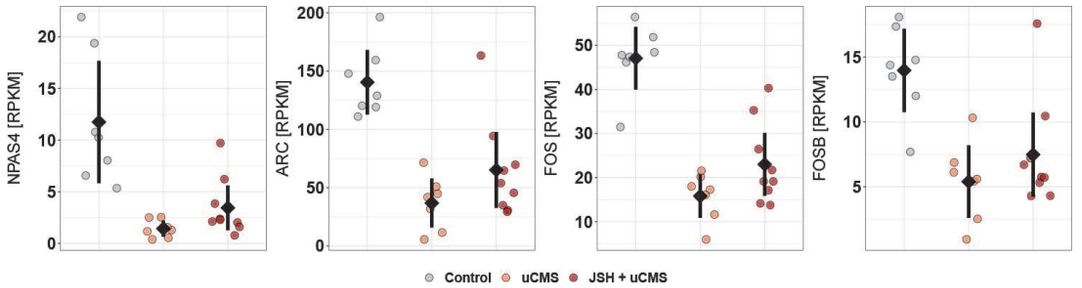

Figure 10.
Animal housing and stress protocols are described in ***Figure 4****. **Experimental details – Next Generation Sequencing:** RNA was extracted using the Ambion Magmax™-96 RNA isolation kit. In brief, cells were lysed (Qiagen Tissuelyzer™), nucleic acids were captured onto magnetic beads, washed, treated with Dnase and eluted in nuclease free water. RNA integrity and concentration were assessed using the Fragment Analyzer (Standard Sensitivity RNA kit, DNF-471, Advanced Analytical). Fifty nanograms of high quality RNA (RIN > 7) were used as input material for the NEBNext Poly(A) mRNA Magnetic Isolation Module and the NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs). NEBNext Adaptors for Illumina were diluted 100 fold prior to cDNA ligation. Adaptor-ligated cDNA was amplified via 14 PCR cycles using NEBNext unique dual index primers (New England Biolabs). PCR products were cleaned up using AMPure XP Magnetic Beads (Beckman Coulter). Libraries were qualitatively and quantitatively assessed using the 1–6000 bp NGS kit (DNF-473, Advanced Analytical) and the Quant-iT PicoGreen dsDNA Assay kit, respectively. Final libraries yields were 40 nM, while fragment size were 350 bp. Libraries were normalized, pooled and clustered on the cBot Instrument using the TruSeq SR Cluster Kit v3 (GD-401-3001, Illumina Inc., San Diego, CA). The clustered flowcells were sequenced on a HiSeq 3000 using a read length of 84 bases in single-read mode, generating an average of 30 million pass-filter reads per sample. **Experimental details – RNA-Seq Data Processing:** Reads were aligned to the reference rat genome (Ensembl 84, http://www.ensembl.org) using the STAR Aligner (2.5.2a). Sequenced read quality was checked with FastQC (0.11.2, http,// www.bioinformatics.babraham.ac.uk/projects/fastqc/) and alignment quality metrics were calculated using the RNASeQC (1.18). Duplication rates of the RNA-Seq samples were computed and marked with bamUtil (1.u.11) and dupRadar (1.4), respectively. Cufflinks (2.2.1) was used to compute the reads per kilobase of transcript per million mapped reads (RPKM) as well as read counts. Normalization factors were calculated using trimmed mean of M-values (TMM) and subsequently reads were voom-normalized. Genes with RPKM values > 5 in at least one group were considered in the final analyses to ensure data quality. The Benjamini-Hochberg's method was used to correct for multiple testing, and only protein-coding genes with adjusted p value < 0.05 were considered as differentially expressed. Pathway analyses were carried out in Ingenuity. **Results:** Reduced expression of immediate early genes after stress in young adulthood. Next-generation sequencing of the PFC was used to quantify gene expression in controls (gray), uCMS alone (peach) and the double-hit group with JSH + uCMS (red). From all significantly different genes, only the most reliable with RPKM > 5 in at least one experimental group were eligible. There was a statistically significant reduction in the expression of Npas4 ($F(2,20) = 13.6$, $p = .0002$), Arc ($F(2,20) = 17.7$, $p < .0001$), Fos ($F(2,20) = 31.2$, $p < .0001$) and Fosb ($F(2,20) = 10.5$, $p = .0008$) in both stress groups compared to controls.*

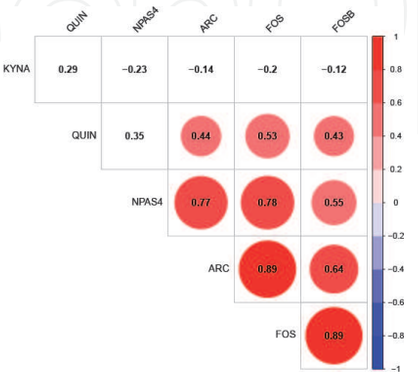

Figure 11.
Animal housing and stress protocols are described in ***Figure 4*** *while methods for TRYCAT profiling and NGS are described in* ***Figures 9*** *and* ***10****, respectively.* ***Results:*** *Correlation of immediate early gene (IEG) expression in the pre frontal cortex (PFC) with plasma levels of the neuroactive tryptophan catabolites (TRYCATs) kynurenic acid (KYNA) and quinolinic acid (QUIN) in 16 weeks old male Wistar Kyoto rats with and without stress exposure during adolescence and / or adulthood. Values represent Pearson's correlation estimate while the presence of surrounding circles indicates statistical significance at a false discovery level of α = .05. Warmer colors mark positive correlations.*

to respond to incoming signals by changing its synaptic outputs [47, 48], was associated with decreased plasma levels of quinolinic acid (**Figures 8–11**, modified from [11]). This suggests that exposure to chronic stress can alter neurotransmission and connectivity in the brain via the kynurenine pathway, which modulates how the current situation is perceived and memorized. Ultimately, stress perception and memory influence how future events will be dealt with. Summed up, profiling of the tryptophan catabolites (TRYCATs) produced in the kynurenine pathway could represent an interesting biomarker for the balance of excitatory and inhibitory neurotransmission, plasticity and learning. TRYCATs may furthermore give insights into processes occurring in the PINE system in response to (chronic) stress, such as the presence of inflammatory processes.

4. Perturbations in the PINE network transition to disorder

The strong inter-connectedness of psychology, immunology, neurology and energy metabolism in the PINE network is very cost and time effective (**Figure 12**, modified from [11]). While the secretion of glucocorticoids as universal messengers in this system is seemingly unspecific, their pleiotropic effects on physiology are well regulated. The fine-tuning is implemented by complex combinations of ultradian GC levels at the event of challenge, the medium-term history of diurnal GC rhythmicity influencing enzyme and receptor expression levels, and the long-term evolved adaptations of PINE component connectivity incorporating the lifetime history of (stress) challenges. In acute stressful situations that decide over life and death, quick and pronounced stress responses are beneficial. However, sola dosis facit venenum and too frequent or much stress can be detrimental for health. The presence of a certain level of GC resistance is a common symptom of stress-associated medical conditions [49]. Resistance of PINE network components to their universal messenger would impede their effective communication. It is thus not surprising that GC resistance in the diseased state is featured with dysregulated immune processes, metabolism and cognition. Regarding the immune system, altered inflammatory signaling has been observed together with glucocorticoid resistance. Respiratory diseases, cardiac disorders, arthritis and inflammatory bowel disease all share a systemic low-grade inflammation associated with chronic stress exposure and altered GC signaling [50]. This chronic low-grade immune activation is not only discussed as feature of somatic disorders, but is as well studied as part of the pathophysiology of depression [51], which itself is a common comorbidity in the aforementioned disorders.

A further entry point for stress to alter the immune system functioning is via energy allocation. The role of bioenergetics has been shown for several aspects of immune system functioning and discussed in the context of therapeutic interventions [52]. Metabolism is not only influencing the activation and proliferation of immune cells [53] but mitochondria are also important for the inflammatory response [54] and regulation of the innate immunity via sensing of danger associated molecular patterns, the inflammasome and ROS-mediated oxidative signaling [55]. Limited mitochondrial capacities to respond to GCs would thus impede immune system regulation. In line with this mechanism, decreased mitochondrial functioning and evidence of slowed metabolism has been observed in patients with disorders where sterile, low-grade inflammation is a commonly observed symptom [56, 57]. Oxidative stress and the associated accelerated biological aging through damage is a likely cause for impaired mitochondrial functioning [29, 58]. Already in the prodromal phase of chronic stress exposure, strong cortisol responses to acute stressors were associated with oxidative stress, suggesting that stress exposure

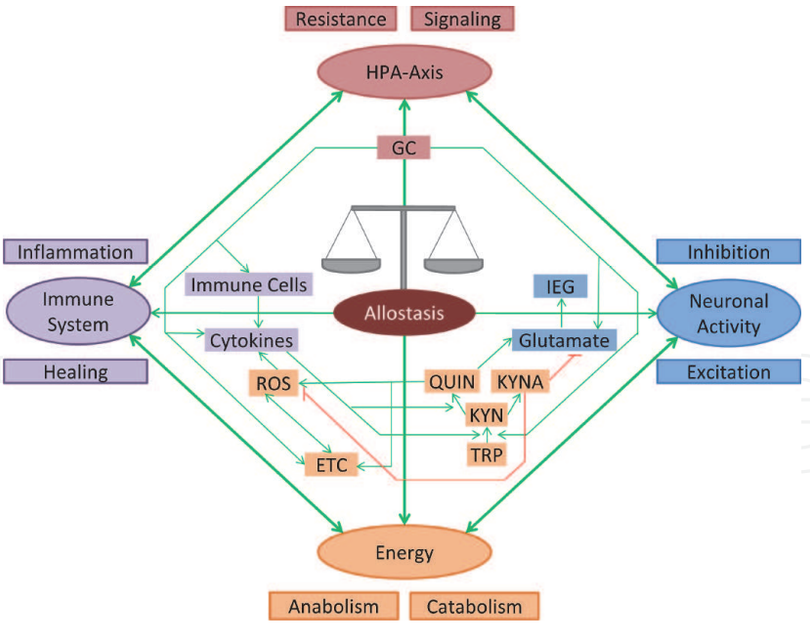

Figure 12.
Networking of the psycho-immune-neuro-energy system to balance allostatic load in response to stress.

promotes oxidative damage through frequent and sustained activation of the HPA-axis [59]. Interestingly, the excitatory neurotransmitter glutamate was shown to contribute to increased mitochondrial ROS production via a TSPO- and calcium-dependent mechanism, which adds to its excitotoxic potential [34]. In depressed patients, the loss of glial and neuronal cells in the PFC, amygdala and hippocampus has been observed [60–62]. Rumination of adverse thoughts in depression and strengthening of the fear-network in post-traumatic stress disorder could reflect enhanced memory recall based on increased hippocampal activity, which could explain the loss of cell density in later stages of the disease. Notably, timing and brain region seem to distinguish whether neuronal activity is beneficial or adverse. Increased hippocampal activity has been associated with stress and pathology, presumably given its sensitivity to compromised energy metabolism that might occur in the aftermath of chronic stress [63]. In contrast, synaptic weakening in the PFC has been associated with resilience to stress, which might be due to increased flexibility in the responsiveness to stress when response patterns are less fixed [48]. However, too few inhibitory outputs of the PFC to the hippocampus may lead to excess hippocampal activity and the resulting over-encoding of stress memory. Finding the right balance of excitatory and inhibitory signaling thus is essential for adequate stress responses and health. In psychiatric patients, this balance was reported to be disturbed [64]. Importantly, 'balance' does not mean a stable level, since the body needs to be able to respond to changes in its environment. As such, the system is never in balance during life but the ratio of excitation and inhibition oscillates following a diurnal rhythm alike glucocorticoids [65]. The need for rhythmicity in excitation and inhibition as well as GC levels is directly linked to the need of flexibility in the stress response system. Resiliency to stress is associated with a highly variable, adaptive capacity. This high degree of freedom in responsivity is key to evolutionary success in terms of fitting to constantly changing environments. Given that the specificity of glucocorticoid signaling is gained by its time and dose-dependency, attenuation in glucocorticoid rhythmicity in response to allostatic load would limit the fine-tuning of PINE network components. Decreased sensitivity or even resistance to GCs would limit their effective communication further. Likewise, the likelihood for persistency of taken adjustments would increase while the adaptive capacity of the PINE network would be reduced. This illustrates the clinical

relevance of GC rhythmicity besides the role in sleep–wake regulation, plasticity or in the context of neurodegenerative disorders. Thus, monitoring the HPA-axis to effectively identify and treat many stress-related chronic illnesses begins to be part of the prevalent practice in the clinics. To fully access functionality, tracing of circadian rhythmicity and the cortisol-awakening response in addition to determination of the responsiveness of the HPA-axis is performed. The latter can be done using the Trier-Social-Stress-Test as challenge or by injecting dexamethasone, a synthetic glucocorticoid, to measure its suppressive effects on the HPA-axis. Patients with psychiatric disorders often show prolonged stress responses after challenge and less inhibition of ACTH and cortisol release when compared to healthy controls [66].

5. Conclusion

Taken together, altered GC signaling is a fundamental symptom in psychiatry that via its communicator role in the PINE network could explain certain other aspects of the diseased state like a pro-inflammatory milieu, compromised energy metabolism or changes in cognition. Whether the transition to disorder originates from this or the other components of the PINE network remains to be further elucidated. Presumably, disease development can not be explained by answering this linear hen-egg-problem but rather requires the joint integration of simultaneous alterations in all components. Therefore, no sequence of events that lead to disorder might be found, but rather patterns of local transitions [67]. These might differ from individual to individual based on the personal life experiences, genetic predisposition, and the surrounding environment. Moreover, the comorbidity of psychiatric and somatic disorders following chronic stress might suggests that maladaptive changes in the PINE network represent a shared prodromal stage in the etiology of these medical conditions. Our understanding of the mechanisms leading to pathology is far from complete and explanations to all facets of the disease remain to be discovered by holistic studies that consider the networking of psychology, immunology, neurology and energy metabolism.

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Conflict of interest

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Abbreviations

ACTH	adreno-cortico-tropic hormone
ATP	adenosine tri phosphate
CNS	central nervous system
CRH	corticotropin-releasing hormone
EAAT	excitatory amino acid transporters
ETC	electron transport chain
FKBP5	FK506 binding protein 5
GC	glucocorticoid
GR	glucocorticoid receptor
HPA	hypothalamus pituitary adrenal
HSP	heat shock protein
IDO	indolamine di oxygenase
KAT	kynurenine amino transferase
KMO	kynurenine mono oxygenase
LTP	long term potentiation
MR	mineralocorticoid receptor
mtDNA	mitochondrial desoxyribonucleic acid
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	N-methyl-D-aspartate
OXPHOS	oxidative phosphorylation
PFC	pre frontal cortex
PINE	psycho immune neuro energy
ROS	reactive oxygen species
TDO	tryptophan dioxygenase
TRYCAT	trypotophan Catabolite
TSPO	translocator protein

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