From Receptor Balance to Rational Glucocorticoid Therapy

E. Ron de Kloet

Department of Medical Pharmacology, Leiden Academic Centre for Drug Research, Leiden University and Department of Endocrinology and Metabolism, Leiden University Medical Center, 2300 RA Leiden, The Netherlands

Corticosteroids secreted as end product of the hypothalamic-pituitary-adrenal axis act like a double-edged sword in the brain. The hormones coordinate appraisal processes and decision making during the initial phase of a stressful experience and promote subsequently cognitive performance underlying the management of stress adaptation. This action exerted by the steroids on the initiation and termination of the stress response is mediated by 2 related receptor systems: mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). The receptor types are unevenly distributed but colocalized in abundance in neurons of the limbic brain to enable these complementary hormone actions. This contribution starts from a historical perspective with the observation that phasic occupancy of GR during ultradian rhythmicity is needed to maintain responsiveness to corticosteroids. Then, during stress, initially MR activation enhances excitability of limbic networks that are engaged in appraisal and emotion regulation. Next, the rising hormone concentration occupies GR, resulting in reallocation of energy to limbic-cortical circuits with a role in behavioral adaptation and memory storage. Upon MR:GR imbalance, dysregulation of the hypothalamic-pituitary-adrenal axis occurs, which can enhance an individual's vulnerability. Imbalance is characteristic for chronic stress experience and depression but also occurs during exposure to synthetic glucocorticoids. Hence, glucocorticoid psychopathology may develop in susceptible individuals because of suppression of ultradian/circadian rhythmicity and depletion of endogenous corticosterone from brain MR. This knowledge generated from testing the balance hypothesis can be translated to a rational glucocorticoid therapy. **(*Endocrinology* 155: 2754–2769, 2014)**

Facing the enormous amount of data generated today by the genomic revolution and real-time imaging technology, where do you start to examine century-old questions such as: What is stress? Does stress cause disease? Is there a future for medicine targeting stress regulation? (Table 1).

My research concerning these questions started more than 40 years ago, and I was thrilled when the first results were selected for presentation at the 1974 edition of The Endocrine Society Meeting in Atlanta. The Symposium was held just before closure of the meeting, and my talk was about the brain glucocorticoid receptors that had been discovered a few years before by Bruce McEwen (1). Besides the chairman and the 3 other speakers, there was 1 other attendant, who fired a snappy question in Franglais which I unfortunately did not understand, not even after the third time it was repeated. The chairman then said: "The question was: did you also study the binding of aldosterone? And your answer is: No." He then proceeded to announce the next speaker leaving me somewhat "lost in translation." But the question about aldosterone binding was highly relevant, because at the time, our notion was that in the hippocampal brain region, more than 1 population of corticosteroid-binding sites coexists, which are now

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Abbreviations: ADX, adrenalectomized; B, corticosterone; CBG, corticosteroid binding globulin; CREB, cyclic AMP response element-binding protein; CRF, corticotropin-releasing factor; dex, dexamethasone; F, cortisol; FKBP5, Tacrolimus (FK506)-binding protein 5; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; 11βHSD-2, 11β-hydroxysteroid dehydrogenase type 2; MR, mineralocorticoid receptor; mTOR, mammalian target of rapamycin; Pgp, P-glycoprotein; PVN, paraventricular.

Table 1. Definitions

Stress is a state of tension that reflects not so much what happens but rather how one takes it. Selye (184) defined stress as "a state of non-specific tension in living matter, which manifests itself by tangible morphologic changes in various organs and particularly in the endocrine glands which are under anterior pituitary control." A stressor is defined as any stimulus that disrupts cellular "homeostasis" (185) or, on the organismic level, as "a real or interpreted threat to the physiological and psychological integrity" (186, 187). Others restrict stress "to conditions where an environmental demand exceeds the regulatory and adaptive capacity of an organism, in particular in case of unpredictability and uncontrollability" (188, 189).
Most stressful is no information, no control, and no prediction of upcoming events with an uncertain feeling of real or imagined threat. A safe place, social context, and self-esteem help to cope with this severe stressful psychological condition (190, 191).
The stress response indicates the physiological and behavioral adaptations to the stressor. Selye distinguished "specific" responses to deal directly with cellular homeostatic disturbances from organism-wide "non-specific" responses. In retrospect, nonspecific is a misnomer for the central, autonomic, hormonal, immune, and metabolic systems that have the capacity to coordinate and integrate the organism's defense reactions to the stressor. To maintain cellular homeostasis, Cannon (185) proposed that "it is the relative stability, despite environmental fluctuations, of those tissue parameters that are critical for cell survival, *e.g.* nutrient availability, oxygen availability, temperature, pH and ion concentrations." As argued by Day (192), "Cannon also mentioned that other parameters that stayed within a normal range at rest, would lead in case of 'emotional excitement' to 'anticipatory' increases in *e.g.* blood sugar, blood pressure and heart rate that 'put the organism in readiness for meeting the demands which will be made upon it."
Neuroendocrinology. Harris and Jacobsohn demonstrated that such environmental demands trigger neuroendocrine secretions via the brain by using "a common final pathway," the median eminence-portal vessel-anterior pituitary route (193). Because the very same hypothalamic and pituitary peptides that stimulated endocrine secretions also carry potent neurotrophic and behavioral activity, De Wied coined the term neuropeptides in the early seventies (194). The Nobel prize for the discovery of the releasing factors was awarded to Guillemin, Schally, and Yalow (195) in 1977. Vale (196, 197) discovered the structure of the CRF-family of peptides.
Allostasis and allostatic load. To study the role of stress in all aspects of life the allostasis concept was introduced (179): "The concept of allostasis, i.e. maintaining stability through change, describes a fundamental process through which organisms actively adjust to both predictable and unpredictable events. Allostatic load refers to the cumulative cost to the body of allostasis, with allostatic overload being a state in which serious pathophysiology can occur."
Stress concept. With the above considerations in mind, the late Seymour Levine (198) stated a practical concept of operation: "stress is a composite, multidimensional construct, in which three components interact: (i) the input, when the stressor is perceived and appraised, (ii) the processing of stressful information and (iii) the output or stress response. The three components interact via complex self-regulating feedforward and feedback loops with the goal to restore homeostasis through behavioral and physiological adaptations."

known as mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) (2, 3).

In this contribution, I will highlight how the function of this dual corticosteroid receptor system has influenced the neuroendocrinology of stress. I will do this in a historical perspective from the identification of both receptor types to their complementary mode of operation during coping with stress (4–7). Two phases in the acute stress reaction can be distinguished through the combination of evidence from the cellular and systems level with functional magnetic resonance imaging-based network analysis of the human brain (8). Thus, during stress exposure, energy resources that initially support a salience network underlying vigilance, selective attention, and emotional reactivity are reallocated to neuronal networks underlying executive cognitive control (8). I will conclude with recent data demonstrating that the effect of acute corticosteroid exposure changes dramatically after a history of chronic stress and argue that the knowledge of the dual MR:GR system can be exploited for a rational glucocorticoid therapy.

This contribution is written in the awareness that an acute stressor can activate within seconds the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, the latter via a cascade of peptidergic secretions from the paraventricular (PVN) parvocellular corticotropin-releasing factor (CRF) neurons and the anterior pituitary pro-opiomelanocortin-producing-producing corticotrophs that release ACTH to stimulate the adrenocortical secretion of corticosteroid hormones. The HPA axis operates as a closed feedback loop to maintain a given setpoint in circulating corticosterone (B) or cortisol (F) concentrations, ie, the corticostat (9, 10).

In response to a stressor, B and F usually reach a peak in circulating concentrations after 15–30 minutes and return to baseline levels an hour later. Based on this temporal aspect, Sapolsky et al (11) classified the function of corticosteroid actions during acute stress reactions as permissive, stimulatory, or suppressive with the potential to determine the outcome of a subsequent stressor. This elegant analysis had its foundations in Munck's original view (12) that the late secretion of corticosteroids serves to limit the

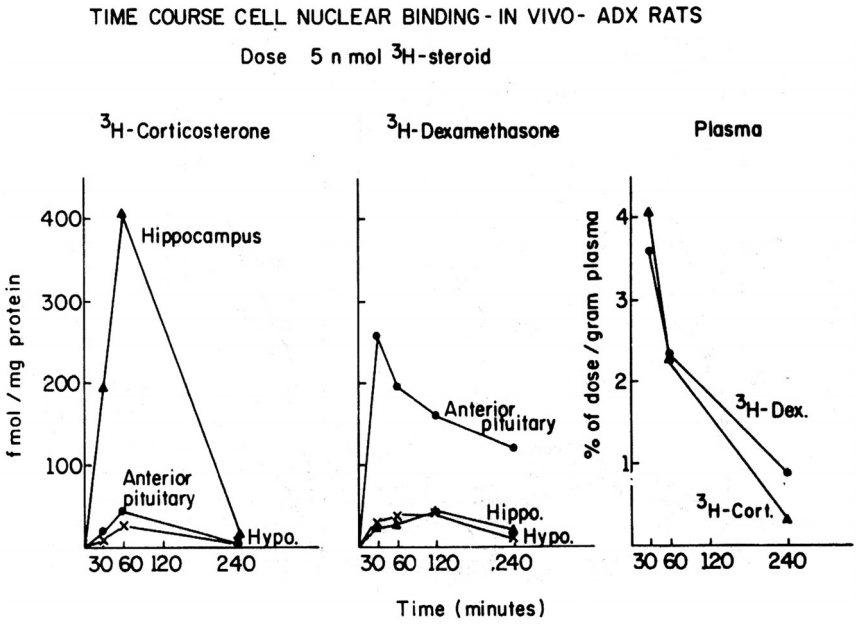

Figure 1. Nuclear retention of ^{3}H-corticosterone and ^{3}H-dex in purified cell nuclei of hippocampus (Hippo), hypothalamus (Hypo), and anterior pituitary at various time intervals after administration in tracer doses to ADX rats. Cort, corticosterone. Reprinted from de Kloet et al (2).

impact of acute stress reactions and to prevent them from becoming overactive and damaging, a concept that is translated here to the endocrinology of the brain. Or as Marius Tausk (1952) in The Netherlands metaphorically stated: "glucocorticoids are required to limit the water damage caused by the fire brigade."

Discovery of Brain Corticosteroid Receptors

December 1, 1968 marked the day I started my PhD research. The day before, on November 30, Bruce McEwen had published in Nature the remarkable finding that tracer amounts of ^{3}H-B were not retained in the hypothalamus and pituitary but rather in cell nuclei of higher limbic brain regions (1). This was remarkable, because at the time, neuroendocrine wisdom dictated that receptors for B would be expected in the core of the HPA axis. Inspired by McEwen's discovery, we decided to use the much more potent synthetic glucocorticoid dexamethasone (dex) for uptake studies in the rat brain. However, despite 3 years of experimentation, we were unable to find dex accumulation in the hippocampus (13). The quest was to discover why.

In 1973, while working as a postdoc in the McEwen lab at The Rockefeller University, we compared using adrenalectomized (ADX) rats in vitro and in vivo the binding of ^{3}H-labeled steroids to soluble receptor proteins and in vivo the retention in a purified cell nuclear fraction of these tissues. We observed that ^{3}H-dex and ^{3}H-F were poorly retained in cell nuclei of the hippocampus, in contrast to the strikingly high retention of ^{3}H-B. In contrast, ^{3}H-dex accumulated in pituitary corticotrophs, its preferential site of action in the suppression of stress-induced HPA axis activity (Figure 1) (2, 3). So there had to be different populations of receptor sites for the corticosteroids in brain and pituitary, a conclusion that was also reached by Rotsztejn after measuring available binding sites in the hippocampus of ADX animals that had received graded doses of B (14).

Meanwhile, also for the mineralocorticoid aldosterone were high affinity binding sites identified in hippocampus resembling those present in the kidney (15, 16). Furthermore, Moguilevski from Roussel Uclaf experimented with the pure glucocorticoid RU26988 and showed that after its inclusion in cytosol, a population of binding sites remained that had not only very high affinity for aldosterone but surprisingly also bound B (17–19). In addition, aldosterone rather than dex could prevent the cell nuclear retention of ^{3}H-B in vivo in the hippocampus (20, 21). These findings suggested that B, like aldosterone, can bind to MR but also, like dex, to GR.

In 1985, Gustafsson and Fuxe (22) presented the first immunocytochemistry of GR, but unlike the high ^{3}H-B retention, the hippocampal cornu ammonis 3 had very low expression of immunoreactive-GR. We then realized that, because the binding affinity of B to GR was 10-fold lower than to MR, the tracer amounts of ^{3}H-B were too low for labeling GR in vivo but sufficient for MR. The nuclear localization of GR required the high circulating B levels that are attained after stress and at the circadian/ultradian peaks (Figure 2) (23). The issue was settled by Evans and coworkers (24), who, after cloning MR and GR, revealed their 94% homology in the DNA-binding domain. MR and GR were proposed to mediate steroid control of overlapping gene networks in binary fashion (25).

The first step in steroid receptor activation involves the reorganization of a cytoplasmic multimeric protein complex and the formation of receptor homodimers that translocate to the nucleus for transactivation, whereas monomers can interact with a variety of transcription factors resulting in transrepression (26). Using fluorescence resonance energy transfer imaging of MR and GR labeled

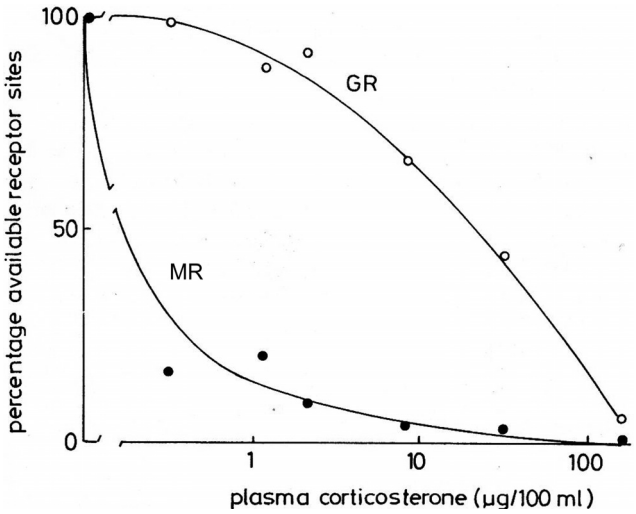

Figure 2. Bioavailability of MR and GR in postmortem hippocampus cytosol of ADX rats at increasing concentrations of circulating corticosterone. MR is determined by Woolf analysis of ^{3}H-corticosterone binding in the presence of a 100-fold excess of the pure glucocorticoid RU26988. GR is determined by Woolf analysis of ^{3}H-RU28362 binding, also a pure glucocorticoid. Data are expressed as % of maximal binding capacity determined in ADX animals (100%). The Bmax of MR and GR is 164.8 and 396.1 fmol/mg protein, respectively. Reprinted from Reul and de Kloet (23).

with different fluorescent proteins, heterodimerization was demonstrated. The data showed that at low corticosteroid concentrations, MR forms homodimers, whereas at higher concentrations mimicking stressful conditions, the formation of MR:GR heterodimers is promoted (27). Heterodimerization is thought to enhance the diversity of corticosteroid actions.

Using confocal microscopy, the colocalization of fluorescent MR and GR in rat hippocampus was observed in distinct domains of chromatin (28). A recent study using chromatin-immunoprecipitation combined with parallel DNA sequencing revealed different ratios of MR and GR binding to DNA that can be altered by the concentration of B (Figure 3) (29). One class likely represents genes implicated in circadian processes, such as *Per1*. Another class was found to respond only to B concentrations occurring during the circadian peak or after stress (29). In microarray analysis, indeed, a few percent of the hippocampal genome appeared responsive to MR:GR activation in distinct and partly overlapping gene networks. An inventory of responsive genes is available (30, 31).

Specificity of MR and GR

But why does brain MR respond to B, while the kidney MR responds selectively to aldosterone in the regulation of sodium homeostasis? In 1988, 2 studies pointed to 11β-hydroxysteroid dehydrogenase type 2 (11βHSD-2) as an enzyme capable in kidney epithelial cells of inactivating F and B but not aldosterone (32, 33). The conversion was blocked by glycerrhetinic acid present in licorice enabling the kidney MR to retain tracer ^{3}H-B, explaining the role of 11βHSD-2 in hypertension (34). Funder and Myles (35) argued, however, that the capacity of 11βHSD-2 was perhaps insufficient to clean the cell of the 100- to 1000-fold excess of bioactive B or F and suggested that the NADH generated by the dehydrogenase additionally caused a redox state unfavorable for MR activation.

The iso-enzyme 11βHSD-1 with cofactor NADPH is widely expressed in neurons and glial cells and serves to regenerate bioactive B and F from their inactive congeners. Particularly during aging, the ensuing intracellular corticosteroids are a concern for causing damage and cognitive decline. Seckl and coworkers (36) managed to protect the aged brain from exposure to excess B by genetic deletion or blockade of 11βHSD-1, a finding that may even-

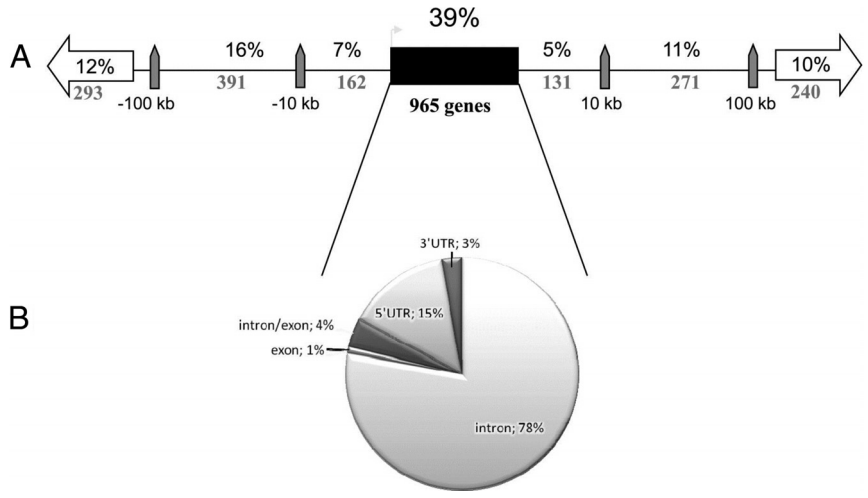

Figure 3. To identify GR binding sites (GBSs) on DNA, corticosterone was administered ip in a high dose of 3000 μg/kg to ADX rats, and after 1 hour, the chromatin-receptor complexes of the hippocampus were precipitated with GR antibodies and the generated DNA fragments subjected to next-generation sequencing (ChIP-Seq). Using this procedure, 2470 significant genomic GBSs were identified at a 13% false discovery rate cutoff. A, Distribution of GBS relative to the nearest gene, resulting in regions that lie within or outside genes. The black bar represents a gene, showing that 39% of the GBSs are located within genes. The GBSs that are located up or downstream from the nearest gene are divided into 3 bins: within 10 kb, between 10 and 100 kb, and more than 100 kb from a gene. B, Pie chart showing the location of intragenic GBS within annotated RefSeq genes, divided into 3′ and 5′-untranslated regions, intron, exon, intron/exon overlap. Reprinted from Polman et al (29).

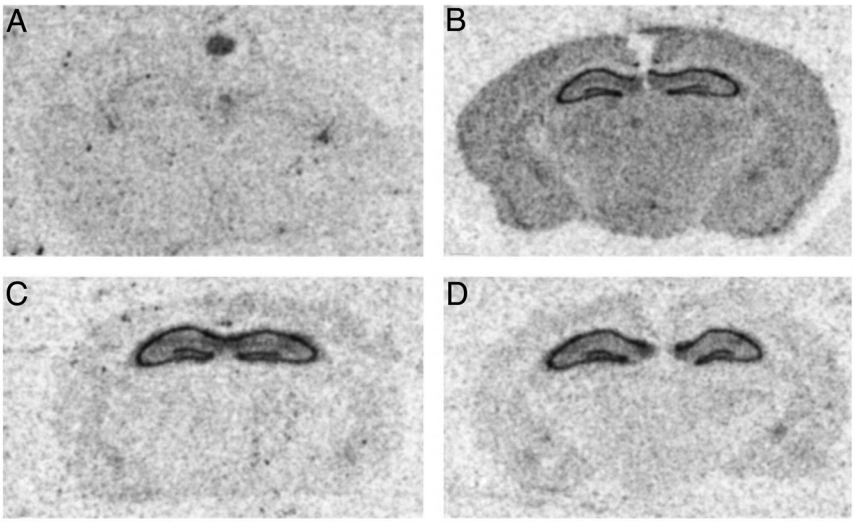

Figure 4. Multidrug resistance Pgp hampers penetration of exogenous cortisol, but not endogenous corticosterone, in mouse brain. Representative autoradiograms of 12-μm coronal sections of the brain of wild-type (A and C) and mdr1a−/− mice (B and D) at hippocampus level. Autoradiograms show labeling with ^{3}H-cortisol (A and B) or ^{3}H-corticosterone (C and D) administered to ADX mice. Note the pituitary mounted on top of the brain. The dark spots in A represent transverse sectioning of the cerebroventricular space and adjacent ventricular walls. A similar pattern as cortisol in wild types and mutants is demonstrated with the synthetic glucocorticoids, such as dex (39). Reprinted from Karssen et al (199).

tually lead to a neuropharmaceutical intervention strategy to manage unwanted effects of cellular hypercortisolemia (37).

But why was the high affinity of dex to GR not been reflected in a distinct cell nuclear retention pattern in the brain as we observed in pituitary corticotrophs? This mystery was solved when it was discovered that dex is a substrate for the multidrug resistance P-glycoprotein (Pgp) transporter (38). We found that a tracer ^{3}H-dex, administered to mice with a genetic deletion of Pgp, showed a pattern of cell nuclear labeling that was reminiscent of the immunocytochemical distribution of GR (Figure 4) (39). This finding has had important implications. Because dex inhibits HPA axis activity, the level of circulating B will be very low, leading to depletion of the steroid from brain MR and GR, a condition we termed chemical ADX (2, 3). Moreover, because dex poorly penetrates the blood-brain-barrier the few dex molecules that can enter will occupy GR rather than MR. Hence, dex treatment produces a condition of severe MR:GR imbalance, because MR becomes unoccupied.

The preferential targeting of the pituitary corticotrophs is fundamental for the dex suppression test launched by Carroll in the early 1970s (40) and Holsboer's more refined dex-CRF test (41). In their book *Endocrine Psychiatry: Solving the Riddle of Melancholia*, Shorter and Fink give an in-depth account of the rise and fall of the dex suppression test in endocrine psychiatry (42).

Thus, the GR is expressed ubiquitously in neurons and glial cells with highest concentration in the PVN, hippocampus, amygdala, cortical regions, and the ascending aminergic neurons. The MR occupied by F or B has a more restricted distribution with highest expression in limbic structures, ie, hippocampus, lateral septum, amygdala, and in discrete sensory and motor neurons (25, 43–45). The distribution of aldosterone-selective neurons expressing 11βHSD-2 is limited to periventricular areas and the brain stem nucleus tractus solitarii (46), areas that are involved in salt appetite, osmotic control, and volume regulation (47, 48).

Ultradian and Circadian Rhythms

B and F display, under basal conditions, an hourly ultradian rhythm, and pulses have their largest amplitude at the start of circadian activity. This is known for several decades (49), but in recent years, Lightman et al (50) have explored its implications in more depth. The pulse pattern seems an intrinsic property of the pituitary-adrenal axis, because oscillations are triggered in any system with a feedback delay (51). The pulse patterns in blood are reflected by oscillations of free B in sc fat and brain (52). The stress response is superimposed on the ultradian rhythm and appears most pronounced when occurring during the ascending arm of the hourly pulse (53). The implication of the hourly B pulses for MR and GR is as follows.

First, the affinity to B is high enough to keep MR in the nucleus over the interpulse interval (54). The mostly nuclear localization is thought to contribute to B's role in maintaining the tone or threshold of HPA axis activity (4). Supporting evidence for this view came from the replacement of ADX animals with graded doses of B (55) and after administration of an MR antagonist (56).

Second, the pulsatility is needed to maintain responsiveness to the circulating corticosteroids (54, 57), as is reflected in the nuclear dynamics of GR, which translocates to the nucleus in parallel with the ultradian rhythm of B (57). Such a mechanism of gene pulsing warrants rapid responding to changing B levels, and indeed, we found desensitization of physiological regulations and be-

havioral responses upon exposure to stable rather than pulsatile B concentrations (54).

Third, the amplitude and frequency of the pulses can change during stressful and disease conditions. Aging is characterized by the disappearance of the pulsatile pattern, whereas pulsatility is completely suppressed by synthetic glucocorticoids. In contrast, chronic exposure of the brain to antiglucocorticoids actually enhances the amplitude of circadian B oscillations (58).

Stress Response and Negative Feedback

Ingle (1938) discovered that corticosteroids can exert a negative feedback action in the HPA axis (59). Where and how this negative feedback is exerted has been the focus of decades of research. Recently, Schmidt et al (60) examined HPA axis activity of mice carrying a conditional knockout of the GR gene specifically targeted at the pituitary corticotrophs. Surprisingly, in adulthood, the HPA axis activity and circulating B levels in the pituitary GR(−/−) mutant were not different from their intact controls. This suggests that a pituitary feedback site of B is less prominent in adult HPA axis regulation, possibly because corticosteroid binding globulin (CBG) present in pituitary prevents B from reaching GR (61–63). Dex does not bind to CBG and can bind to pituitary GR to exert potent inhibition of HPA axis activity (13, 64).

In the mouse PVN parvocellular neurons, the deletion of GR-exon 3 caused a profound (87%) reduction of immunoreactive-GR expression. In contrast to the pituitary GR(−/−), these PVN GR(−/−) showed in adulthood elevated ACTH and B levels during the circadian peak and in response to a restraint stressor (65). The mutants were also resistant to dex suppression. One reason for this resistance may be the deletion of GR feedback site in the PVN. Alternatively, dex acting in the pituitary might not have overcome the strong hypothalamic drive for ACTH release, an issue that can be resolved by a dex dose-response study. The PVN GR mutants did not display an anxiety or depressed phenotype despite their high circulating B.

Although the mutant study points to the PVN as a primary feedback site for B, a recent study using ChIP failed to show binding of GR near the *Crf*-promoter, whereas phosphorylated cyclic AMP response element-binding protein (CREB) did bind (66). In cultured hypothalamic IVB cells, dex treatment induced association of GR with histone deacetylase 1 and methyl CpG binding protein and increased the occupancy of the *Crf*-promoter by these proteins and DNA-methyltransferase 3b (67). In accordance with these findings, dex treatment increased promoter methylation at specific CpG sites and histone 3-lysine 9 residues, which can subsequently repress CRF transcription. Furthermore, in a chronic social stress paradigm, demethylation of the DNA-CREB-binding sites was found associated with enhanced CRF transcription but only in those animals that also showed social avoidance to an unfamiliar mouse. These studies by Elliott et al (68) convincingly demonstrated that epigenetic regulation of CRF expression may be a primary molecular mechanism underlying stress-induced neuroendocrine changes.

These recent data provide support for the concept of complementary levels of corticosteroid feedback. First, fast rate-sensitive feedback operating within minutes (69–72). Second, an intermediate feedback mechanism taking 30 minutes to a few hours involving an action of corticosteroids on afferent pathways that project to the PVN (71, 73). Third, a slow- and long-lasting feedback that seems more concerned with regulation of the HPA axis setpoint involving recruitment of methyltransferases and histone (de)acetylases by corticosteroids in the PVN (67, 68), and possibly also elsewhere in the brain (74–76). Fourth, a putative emergency brake at the pituitary level, which is a target for high B levels exceeding CBG capacity as well as dex (Figure 5) (13, 60).

MR:GR Balance Hypothesis

The MR:GR balance hypothesis predicts that "upon imbalance in MR:GR regulated downstream limbic-cortical signaling pathways the initiation and termination of the stress response is compromised. This may lead to a condition of HPA axis dysregulation and impaired behavioral adaptation, which can enhance susceptibility to stress-related neurodegeneration and mental disorders" (5–7, 77).

In this hypothesis, MR and GR operate in complementary fashion in control of adaptation to environmental demands: MR, GR, and their downstream partners not necessarily are in fixed equilibrium but may change in response to environmental demands. Stress immediately activates the central and peripheral components of the sympathetic nervous system. In this context, activated MR modulates in limbic structures appraisal processes and retrieval of stored information that is at the root of taking decisions in crucial questions underlying the onset of a stress and emotional reactions, such as: is this individual a friend or a foe? is this situation a threat or will it provide a benefit? GR is involved in the redistribution of energy resources towards limbic-cortical networks underlying the management of later adaptations, which collectively signal the off-button of the stress reaction (Figure 6) (7, 8, 78).

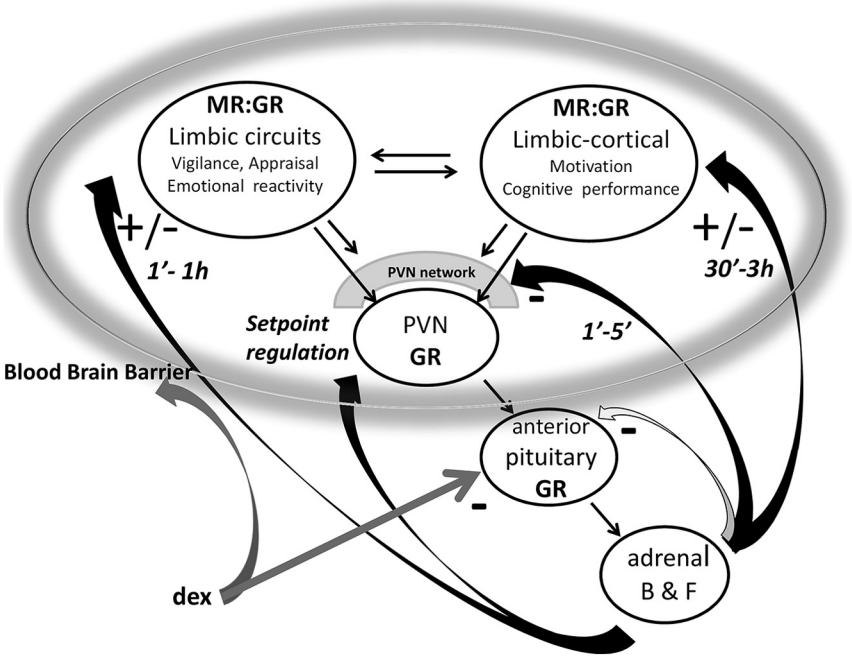

Figure 5. Schematic view of B (rodent), F (man), and Dex action in brain. The effects of B and F are mediated in complementary fashion by MR and GR in various brain circuits. First governed by MR rapidly in limbic circuits involved in vigilance, appraisal, and emotion regulation and next with rising hormone concentrations via GR in limbic-cortical regions involved in reward- or fear-motivated behavior and cognitive performance. B and F also exert a rapid feedback actions on an inhibitory network surrounding the PVN. The actions in the PVN rather is involved in setpoint regulation. Dex poorly penetrates the brain and targets the pituitary to suppress stress-induced HPA axis activity and thus B/F secretion from the adrenals. As a consequence, dex suppresses the ultradian and circadian rhythms and depletes in particular the brain MR of endogenous hormone and alters the MR:GR balance. During repeated exposure to stressors, the MR:GR balance may change and, thus, the balance between emotion and executive functions, with consequence for mental health (based on data from Refs. 2, 8, 23, 39, 56, 65–72, 82, 83, 86, 93, 101, 104, 117).

Fundamental for the hypothesis are the cellular response patterns to steroid exposure documented by Joëls et al (79–81). Key is the discovery of the membrane variant of MR that rapidly enhances excitatory transmission by a nongenomic mechanism stimulating the presynaptic release of glutamate (81–84), whereas via GR, glutamate release and excitation is suppressed (85, 86). The studies also demonstrate that depending on the concentration of B, cells integrate a response pattern over time domains ranging from minutes to hours. A low steroid concentration during the circadian trough activates nuclear MR, whereas the rising B concentrations after stress and during the ultradian/circadian peaks are needed for nuclear GR and both putative membrane MR and GR types; this pattern of receptor activation is reflected in synaptic plasticity (87–92).

The rapid transient increase in excitability in limbic structures induced by B in concert with other rapidly acting excitatory transmitters and neuropeptides helps the individual to appraise environmental input and to retrieve and to select an appropriate behavioral response (93). The prolonged activation of excitatory transmission the basolateral amygdala achieved by metaplasticity involving cooperation of MR- and GR-mediated cellular mechanism is in line with the prominent role of these circuits in encoding emotional experience (94, 95). Subsequently, at a later time with higher B concentrations and GR activation, the raise in excitability is suppressed, whereas resources are shifted to elsewhere in limbic and frontal cortex regions to promote higher cognitive and executive functions. The fast and slow effects that redistribute energy from circuits underlying attention and vigilance to learning and memory processes are crucial for adaptation to stress (8, 78, 80, 81, 96).

There is an enormous diversity in molecular changes occurring after stimulation of MR and/or GR in the various circuits innervating the PVN, whereas over time, B drives waves of genomic responses (97). These actions exerted by B proceed in concert with the other stress mediators that each operate in their own domain of time, space, and context (79–81). However, as a hormonal signal, B's action is aimed primarily at coordinating these diverse molecular changes and cellular responses to environmental input. Moreover, B has the capacity to integrate these molecular and cellular mechanisms over time by tuning rapid membrane responses and slow genomic regulations (80) with the ultimate goal of maintaining cellular homeostasis and promoting adaptation. MR- and GR-mediated actions are interdependent: information stored for future use via GR activation, either adaptive or maladaptive, is later in an appropriate context retrieved via MR-controlled networks.

Testing the Balance Hypothesis

Support for the balance hypothesis comes from the measurement of the receptors themselves. In human postmortem cingulate cortex, dorsolateral prefrontal cortex, and hippocampus of patients that had suffered from mood disorders, MR expression was significantly decreased if compared with well-matched controls (98–100).

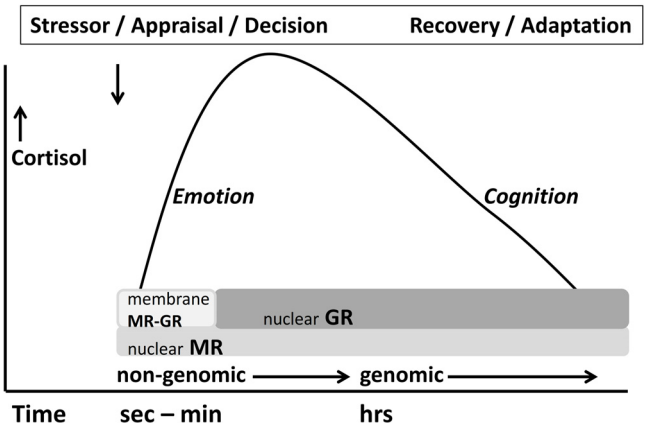

Figure 6. Temporal changes in complementary MR- and GR-mediated action in the brain during the stress response that are initiated by the perception and appraisal of novel stressful events with emotional expressions of fear and aggression involving MR operating in the context of other signaling systems, such as, eg, the sympathetic nervous system. With rising hormone concentrations, energy resources are mobilized to promote recovery and to activate circuits involved in adaptation and storage of the experience in the memory (for future retrieval via MR). In some functions, MR and GR operate independent, for others, such as, eg, emotional expression, MR and GR cooperate, and there are functions where MR and GR mediate opposing actions, such as in the initiation and the suppression of the stress response (based on data from Refs. 7, 11, 71, 80, 81, 83, 93, 96, 117, 200).

In rodents, at the neuroendocrine and behavioral level, genetic modification of MR and GR has provided data that can be interpreted in support of the balance hypothesis. Laryea et al (65) produced selective GR knockouts in the PVN that showed HPA axis activation and a metabolic rather than a behavioral phenotype. Boyle et al (101) generated an animal model with GR expression conditionally disrupted at 4 months of age in forebrain regions, including the hippocampus and basolateral amygdala, but not in the central amygdala, PVN, or pituitary. This mutant showed enhanced HPA axis activity as well as features of depression and anxiety, of which the depressive phenotype could be reversed by antidepressants. Local disruption of the GR gene in the central amygdala (102) or dentate gyrus (103) caused an impaired conditioned fear response.

Harris et al (104) used mice with forebrain MR overexpression and global GR underexpression. A significant interaction was found between MR and GR in the regulation of the HPA axis and some domains of cognitive performance. In neuroendocrine realm, the stress-induced HPA axis activity was enhanced in the GR_{low} mutants. The high forebrain MR expression concomitant with GR underexpression did, as expected, restrain the HPA axis overshoot after stress. The same combination of MR_{high} with GR_{low} produced a phenotype characterized by enhanced perseveration, suggesting enhanced spatial memory and/or reduced flexibility in choosing an alternative behavioral response.

Genetic variants of MR have been identified by DeRijk et al (105), others found GR polymorphisms (106, 107), and Binder (108) discovered that the regulatory protein FKBP5 operates in an ultrashort feedback loop with GR. These genetic variants of Tacrolimus (FK506)-binding protein 5 (FKBP5), MR, and GR were found correlated with risk of depression and the efficacy of antidepressant therapy. Severe stressors, (early) life experiences, or antenatal glucocorticoid treatment also leave their marks on MR:GR and their chaperones through lasting epigenetic modifications (109–112). The research on the impact of (epi)genetic variations on MR:GR functioning is just beginning.

Targeting the MR:GR Balance in Stress Vulnerability

Selye distinguished in the General Adaptation syndrome an alarm, resistance, and exhaustion phase during progressive exposure to stressors. Moreover, in response to a heterotypic acute stressor, sensitization rather than habituation occurs (113). This vicious circle of impaired recovery from stress and higher corticosteroid exposure because of feedback resistance is the basis of the glucocorticoid cascade hypothesis of stress and disease (114). A consequence of chronic stress exposure is that afferents to PVN are overexposed to corticosteroids, causing decreased neurogenesis (115) and atrophy (116, 117) in the hippocampus and parts of the prefrontal cortex. However, in amygdala and orbitofrontal cortex (118), hypertrophy of dendritic arborizations and spines is observed. These are considered structural adaptations to chronic stress.

According to Selye, the imperfections of the adaptation syndrome coincide with an altered balance in adaptive hormones and are important in the pathogenesis of most stress-related diseases. Selye referred in this context to the pendulum hypothesis, where excess mineralocorticoid over glucocorticoid enhanced vulnerability to inflammation, whereas the reverse enhanced risk of infection. Although the pendulum hypothesis is based on 2 adrenal hormones, the balance hypothesis relies on 1 single hormone B (or F) acting as a double-edged sword via MR and GR. During chronic stress, the receptor balance is disturbed, predicting an altered response to an acute stressor or a B challenge.

Indeed, a history of chronic stress increased in the mouse hippocampus the number of genes responding to an acute stressor (119) with a particularly high responsiveness of the cytokine/NFkB pathway (119, 120). Previously, cellular studies had shown increased calcium currents and excitatory transmission as indices of enhanced

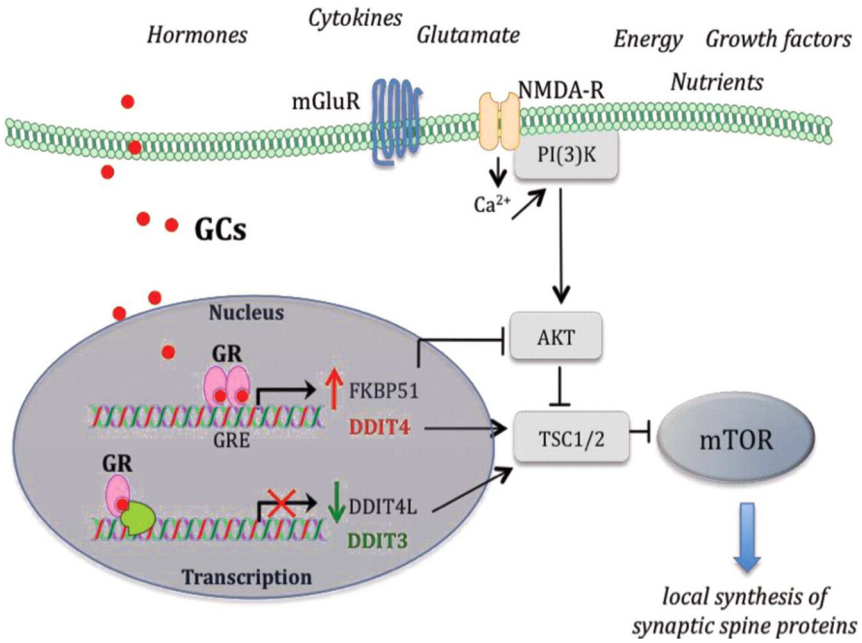

Figure 7. Schematic overview of key components of the mTOR pathway and a number of its physiological and molecular regulators in the brain, indicating a role for GC (glucocorticoids). After GC binding to GR, FKBP51 and DDIT4 are up-regulated by a GRE-driven mechanism, whereas DDIT4L and DDIT3 are down-regulated via a non-GRE-driven mechanism. These mTOR regulators will influence the overall levels of mTOR, with consequences for local synthesis of synaptic spine proteins and thus for synaptic plasticity. DDIT4, DNA-damage-inducible transcript 4 protein; GRE, glucocorticoid response element; DDIT4, DNA-damage-inducible transcript 4 protein; GRE, glucocorticoid response element; PI(3)K, phosphatidylinositol 3 kinase; AKT, v-akt thymoma viral protooncogene 1; NMDA-R, N-methyl-D-aspartate receptor; GluR, glutamate receptor; TSC1/2, tuberous sclerosis protein 1/2. From Polman et al (122).

vulnerability after a history of chronic stress but only if acutely challenged with B (121). In the laser-dissected dentate gyrus (where neurogenesis occurs) of controls, 26 different gene ontology terms could be assigned in pathway analysis, but the diversity in the B-responsive pathways was in the stressed group reduced to 7 (31). After chronic stress, B induced particularly genes involved in chromatin modification and epigenetics (31, 122). One highly responsive gene network revealed by B challenge after chronic stress is the mammalian target of rapamycin (mTOR) signaling pathway, which is critical for different forms of synaptic plasticity and appears associated with depression (Figure 7) (123).

Because B challenge uncovers enhanced responsiveness of dysregulated pathways in limbic regions, it is reasonable to assume that this very same mechanism may also represent a target for treatment. Indeed, by using gene transfer technology, it was demonstrated that enhanced expression of MR locally in the hippocampus (124) or amygdala (125) was protective. Gene delivery of additional 11βHSD-2 (126) inactivating excess B in the hippocampal dentate gyrus reversed its damaging effects. Also, chronically blocking GR with an antagonist improved cognitive performance (127), reversed suppression of neurogenesis, Ca current and long-term potentiation (128), and rescued the CREB signaling pathway (129). Antiglucocorticoid treatment or genetic deletion of GR after chronic stress restored the hyperactive dopaminergic mesolimbic/cortical-amygdala loop and social behavior (130, 131).

Drugs targeting selectively the limbic brain MR are aimed at modulating emotional expressions. First, in a social encounter, it appeared that blocking MR reduced the propensity of aggressive behavior (132, 133). Second, in another paradigm, social interaction was enhanced after either pharmacological MR blockade or forebrain deletion of the MR (134). Third, Schwabe et al (135) demonstrated that stress induced a shift from the use of declarative to habit memory that was prevented by MR blockade with spironolactone, a treatment that also reduced selective attention (136). Functional magnetic resonance imaging showed that amygdala-hippocampus connectivity switches to the caudate nucleus. The data are congruent with animal studies showing a similar MR-dependent stress-induced behavioral and connectivity switch from hippocampus to the caudate nucleus (137–140).

Perspectives

B was identified by Reichstein (1936), who in 1950 received the Nobel prize in Physiology and Medicine jointly with Kendall and Hench, "for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects." Their discovery heralded the treatment of patients suffering from inflammatory and autoimmune disorders with synthetic glucocorticoids, currently for about 1% of people in the Western world (141). However, this treatment causes a 2-fold increased risk of depression, a 4-fold increased risk of mania, delirium, confusion, or disorientation, and nearly a 7-fold increased risk of suicide (141). Moreover, after cessation of excess glucocorticoid exposure, patients may have enduring psychiatric complaints (142). The search for more selective glucocorticoids has had little success, but recently, selective GR modulators were developed (143). One of these new

compounds exerted agonist effects in the suppression of stress-induced HPA axis activity but lacked unwanted stimulatory effects on amygdala CRF (144).

One possible cause of glucocorticoid-induced psychopathology is that blockade of the HPA axis also inhibits the pulsatile secretion of endogenous corticosteroids; the continuous exposure to the synthetic glucocorticoid then causes desensitization and reduced responsiveness of GR-dependent neuronal networks. Pulsatility is also absent in adrenal-deficient patients (145–147). Delivery methods are being developed that should release the steroids according to a circadian pattern.

Another cause of psychopathology may be the severe MR:GR imbalance induced by synthetic glucocorticoids. The profound suppression of endogenous corticosteroids by steroids like dex and prednisone, their poor penetration into the brain, and consequent depletion of B and F from brain MR may also present a health risk (148–150). Indeed, Liston et al (151, 152) recently demonstrated in dex-treated rats that circadian oscillations of B are a prerequisite for learning-dependent synaptic plasticity. Additional intermittent administration of B was needed to maintain balanced dendritic spine formation and pruning in vivo in the cerebral cortex as was demonstrated by live imaging using transcranial 2-photon microscopy. This finding provides proof of principle to supplement glucocorticoid therapy with F in oscillating concentrations.

F might actually be used therapeutically to modulate the processing of stressful information. For such an approach, protocols are needed that account for the precise timing and context of hormone action (80, 153–155). Thus, F disrupted the acquisition or retrieval of information within minutes (156, 157), and if infused during a fear-conditioning paradigm, generalization of fear occurred as in posttraumatic stress disorder (158). These effects are rapid and can be blocked by MR antagonists (92, 159, 160). If F is given at longer time intervals (4 h) before learning memory storage was promoted (158), but when given a few hours after learning memory extinction of a traumatic experience was facilitated (161). These effects can be blocked by antiglucocorticoids, suggesting involvement of GR. Antiglucocorticoids seem useful in conditions where excess F causes brain pathology as in Cushing's disease, psychotic depression, and diabetes (162–164).

A synthetic analog of F with an interesting pharmacological profile is fludrocortisone. Although this compound is clinically mostly used in low doses as MR agonist during adrenal deficiency or postural hypotension, it is actually a potent mixed agonist of both MR and GR (165). When infused in rats, fludrocortisone affected MR-dependent appraisal and risk assessment if given before fear conditioning but promoted fear memory if given immediately after (166). The hyperactive HPA axis of psychotically depressed patients escaped suppression from fludrocortisone as is observed after dex (167). Fludrocortisone was shown to promote sleep-dependent memory activation (168) and stimulated feelings of empathy in female borderline patients (169). Moreover, the steroid enhanced the efficacy of antidepressants in depressed patients (170).

Personalized treatment with glucocorticoids will likely benefit from testing for MR and GR gene variants (171–174). MR haplotype 2 is associated with dispositional optimism and protects against depression (170–172). The GR variant N363S is hypersensitive to F and associated with an unhealthy metabolic profile (107), whereas ER22/23EK is linked to steroid resistance and risk of depression (107). The B*c*/1 polymorphism might be a predictor for side effects of glucocorticoid therapy (107, 174). Currently, trials are underway to exploit this knowledge on gene variant function to the benefit of patients suffering from traumatic memories in posttraumatic stress disorder and other anxiety disorders (174).

Conclusion

An overarching question is how corticosteroid action in the brain can change from protective to harmful. Here, this question was addressed from the perspective that corticosteroids act as a double-edged sword: they enhance over time first rapidly emotional expressions and then in slower fashion cognitive performance underlying stress adaptation. Evidence from the cellular, systems, and neuronal network level suggests that this dual action exerted by the steroids is mediated in complementary fashion by MR and GR. In the context of multiple stress signals, the balance in MR:GR and their downstream signaling pathways has relevance for mental health. A severe MR:GR imbalance also occurs during treatment with potent synthetic glucocorticoids: to correct the balance a suppletion with endogenous F or B at appropriate times to match ultradian and circadian variations could be helpful to optimize glucocorticoid therapy (50, 145–147, 150–152, 168) in the face of adrenal atrophy.

Time and space are important variables in the heuristic value of the MR:GR balance theory for understanding the pathogenesis of stress-related mental disorders. As predicted in the General Adaptation syndrome concept (175), the experience of chronic stress will initially enhance via MR the sympathetic outflow (176) and excitatory transmission (83, 177) in the limbic brain underlying emotional reactivity at the expense of energy for GR-mediated higher

cognitive and executive functions (Figure 6) (7, 8). This state of increased resistance (175) or "allostatic load" (178, 179) is characterized by propensity of anxiety and aggression, which can be attenuated with an MR antagonist (132–136, 139). With further progression, a state of exhaustion may develop, where excess GR stimulation compromises energy metabolism (11, 114), and rather MR agonists (167, 169, 170) or GR antagonists (130, 131, 163, 164) are indicated. This sequence of events was recently qualified as a cortisol-induced, serotonine-dependent, aggression/anxiety-driven subtype of depression (180).

MR:GR imbalance, thus, appears associated with dysregulated HPA axis activity, which is a hallmark for stress-related mental disorders. Unraveling the precise role of each receptor may help, therefore, to understand mechanisms of vulnerability and resilience in the diseased brain. This is not trivial, because an intrinsic property of stress sensitization is chromatine reorganization underlying lasting changes in brain circuits, a phenomenon that can be uncovered by acute challenge with a stressor or a glucocorticoid (31, 120). One direction to make progress in the treatment of psychopathology would be to identify (epi) genetic markers for individual-specific susceptibility pathways leading to disease (181) that can be examined using translational endpoints in humanized models (177, 182, 183). To address this from a translational perspective, an understanding is needed of clinical and functional phenotypes, life histories, and (epi)genotypes of the individual.

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Address all correspondence and requests for reprints to: E. Ron de Kloet, PhD, Department of Medical Pharmacology, Leiden University and Department of Endocrinology and Metabolism, Leiden University Medical Center, Gorlaeus Laboratory, PO Box 9502, 2300 RA Leiden, The Netherlands. E-mail: erdekloet@gmail.com.

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