# Stress Research: Past, Present, and Future

## Abstract

This chapter starts with highlighting the evolution of the stress concept and the discovery of mediators that coordinate stress adaptation. Next, progress in the unraveling of the mechanism underlying the action of these stress mediators is discussed, focusing on glucocorticoids as the end product of the hypothalamic pituitary adrenal (HPA) axis. This action exerted by the glucocorticoids is mediated by a dual receptor system: mineralocorticoid (MR) and glucocorticoid receptors (GR). With these receptors as leading theme we present five highlights that illustrate the serendipitous nature of stress research. These five highlights are integrated in the final section which culminates in reflections on the role of stress in mental health. In these reflections we merge the mind-boggling complexity of molecular signaling pathways with neuroendocrine communication integrating body and brain functions. The new insights will be used during the next decennium to target, in an individual-specific fashion, the stress system with the objective to enhance the quality of life.

Abbreviation

ACTH Adrenocorticotropic hormone - ADX Adrenalectomy - APO-SUS Apomorphine-susceptible - B Corticosterone - BLA Basolateral amygdala - CRH Corticotropin releasing hormone - Dex Dexamethasone - ERK Extracellular regulated kinase 1/2 - F Cortisol - GR Glucocorticoid receptor - HPA axis Hypothalamic-Pituitary-Adrenal axis - 5-HT 5-Hydroxytryptamine = serotonine - 5-HTT Serotonin transporter - LTP Long-term potentiation - MR Mineralocorticoid receptor - mdr Multidrug resistance - mEPSC Miniature excitatory postsynaptic current - POMC Pro-opiomelanocortin - PPI Prepulse inhibition - PVN Paraventricular nucleus - SHRP Stress hyporesponsive period - SNP Single nucleotide polymorphism

## Brief History

### From Stress Concept to Allostatic State

From a historical perspective, Cannon linked as early as in 1915 the sympathetic mediator adrenaline with the fight, flight, or fright response to cope with a threat, a notion that marks a first step in the contemporary evolution of the stress concept (Table 64.1). The end products of the hypothalamus-pituitary-adrenal (HPA) axis, that is, the glucocorticoids cortisol (F, in man) and corticosterone (B, in rodent), were first synthesized in 1936 by Reichstein and Laqueur. Ever since, glucocorticoids are tightly linked to stress, the term coined by Hans Selye in the same year to describe the "nonspecific reaction of the body to noxious stimuli" (Table 64.2). What stress actually is, always spurs vigorous debates. We favor the view of one of the pioneers in stress research, the late Seymour (Gig) Levine who defined "stress" as a composite, multidimensional construct, in which three components interact: (1) input, when the stressor is perceived and appraised, (2) processing of stressful information, and (3) output or stress response. The three components interact via complex self-regulating feedback loops with the goal to restore homeostasis through behavioral and physiological adaptations. These adaptations need to be coordinated in brain and body; the major communication systems, the autonomic nervous system and the HPA axis, are extremely important in this respect.

Table 64.1 Evolution of the stress concept

| | | |
|---|---|---|
| Claude Bernard | 1850 | Homeostasis |
| Walter Cannon | 1915 | Fight/flight/adrenalin |
| Hans Selye | 1936 | Stress/cortisol |
| John Mason | 1968 | Experience stressor |
| Jay Weiss | 1972 | Coping with stressor |
| Sterling/McEwen | 2000 | Allostasis |

Table 64.2 Milestones in glucocorticoid research

| | | |
|---|---|---|
| 1855 | Addison | Addison's disease |
| 1856 | Brown Sequard | Adrenals indispensible for life |
| 1936 | Kendall, Laqueur | Discovery corticosterone = glucocorticoid |

| | | |
|---|---|---|
| 1936 | Selye | Glucocorticoids linked to stress |
| 1938 | Ingle | Feedback glucocorticoids demonstrated |
| 1950 | Kendall, Reichstein, Hench (nobel prize) | Cortisol relieves rheumatoid arthritis |
| 1952 | Tausk, Munck 1984 | Cortisol protects against primary stress reaction |
| 1968 | McEwen | Corticosterone receptors in brain |
| 1985 | Evans | Cloning glucocorticoid and mineralocorticoid receptors |
| 1985 | De Kloet & Reul | Mineralocorticoid (MR) and glucocorticoid receptors (GR) in brain |
| 1995 | Karin | Glucocorticoid transrepression vs transactivation |
| 2005 | Tasker/Karst/Joels | MR and GR action at membrane |
| >2005 Genetic & epigenetics of MR & GR; MR & GR balance risk factor for mental disease, co-regulators of MR & GR, micro-RNA's, glucocorticoid responsive gene pathways | | |

Selye called this effort of the organism to adapt to noxious stimuli the "general adaptation syndrome" and distinguished during the course of exposure to stressors an initial phase of alarm, then over days or weeks a phase of resistance in which the individual seemingly coped with the chronic stressor and finally exhaustion, a phase characterized by breakdown of adaptation. While Selye focused mainly on the stressor and the (patho) physiology of the stress response, the research of Levine (2005) and others emphasized that stress is about the processing of the individual experience of the stressor and the ability to cope. Thus, the most severe stressor is a psychological condition characterized by lack of information to predict upcoming events, with no sense of control and with an uncertain anxious feeling of threat, either real or imagined.

The ability to cope with such a psychological stressor is dependent on experience- and gene-related factors, and is affected by cognitive, noncognitive, and environmental inputs. Moreover, coping resources rely on the context in which the stressor is experienced. Powerful determinants of context are psychosocial factors such as social position, social support, or attachment to a caregiver. If any of these factors is disrupted, for example, loss of control in a social environment, expulsion from social support, homelessness or deprivation of (maternal) care - an acute stressor may exceed the coping resources and produce strong emotional reactions, which ultimately may lead to a condition of chronic stress, exhaustion or burnout, and enhanced vulnerability to mental diseases such as depression or anxiety disorders.

These modulations of the stress response have been defined by McEwen and Wingfield (2010) as variations in an allostatic state that cumulatively strive toward homeostasis; allostasis being defined then as the process to reestablish homeostasis through changing allostatic states. In principle, these changing allostatic states are adaptive, self-preservative, and short-lasting. In terms of communication, successful allostasis (in establishing homeostasis) would mean,for example, that the HPA axis hormones involved are turned on rapidly when needed and turned off efficiently when homeostasis has been achieved. The hormonal responses however may be inadequate, or excessive and prolonged and the cost to maintain homeostasis may become high. This leads to wear and tear, or allostatic load, ultimately enhancing the vulnerability to disease. At a behavioral level, for instance, depression may be interpreted as a consequence of sustained hyperactivity of CRH and the sympathetic nervous system, and excess circulating glucocorticoids.

# Introduction

## Basal Pulsatility and Stress Adaptation

Geoffrey Harris established that peptides from the hypothalamus reach the pituitary gland via the portal vessel system in the pituitary stalk. For the actual identification of these releasing factors controlling the synthesis and release of the pituitary hormones, Guillemin and Schally were awarded the Nobel Prize in 1977. Yet, it lasted until 1981 when Wylie Vale identified corticotrophin-releasing factor (CRH) that is synthesized in the paraventricular nucleus (PVN) of the hypothalamus. CRH synergizes with vasopressin in promoting the synthesis and release of ACTH cleaved from the pro-opiomelanocortin (POMC) precursor which in turn stimulates the secretion of B or F from the adrenals. The glucocorticoids feed back on the brain to shut off their own stress-induced secretion and therefore operate in a closed feedback loop first demonstrated in a classical experiment by Dwight Ingle (1938) (Fig. 64.1).

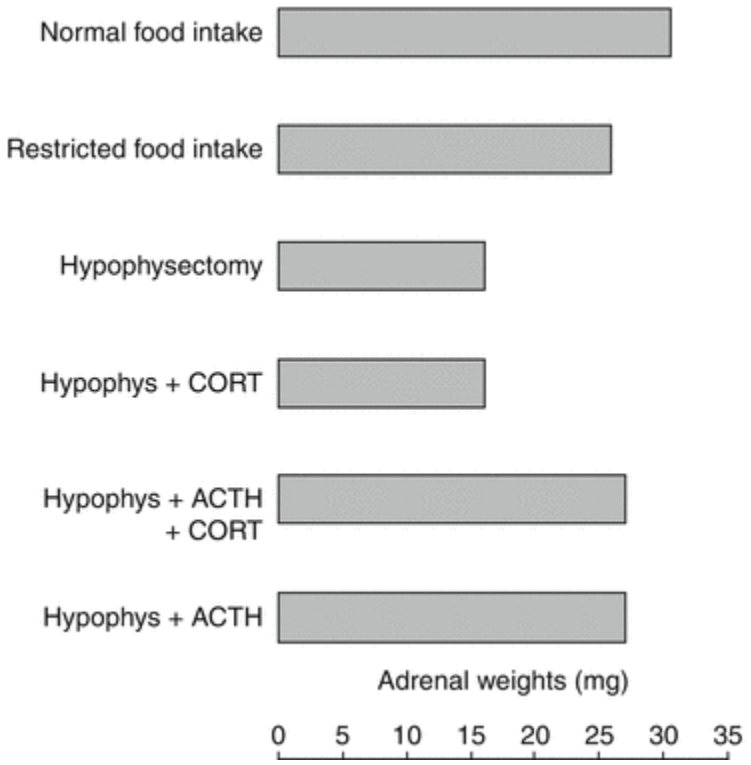


Fig. 64.1 Dwight Ingle: Discovery of feedback (Modified from Ingle DJ (1938). Am J Physiol 124: 369-371). Hypophysectomy, removal of pituitary gland; CORT purified adrenal extract containing corticosterone administered via the drinking water, ACTH injection of purified pituitary extract containing ACTH

Perhaps, one of the most influential concepts developed by Harris was that neuroendocrine systems (such as the HPA axis) are capable of coordinating experience and behavior with the secretion and action of hormones (Fig. 64.2). In the behavioral realm of this concept, David de Wied (1925-2004) coined the term "neuropeptide" in the early 1970s by demonstrating the potent central actions in fear conditioning paradigms of oxytocin, vasopressin, and ACTH or their fragments devoid of classical endocrine activity. Vasopressin, ACTH-related peptides, and CRH promote memory of such fearful experiences, while oxytocin is amnestic. In subsequent studies, discrete patterns of oxytocin, vasopressin, and their receptors were identified in the brain: the peptides appeared crucial in coordinating cognitive functions with socio-reproductive patterns of behavior. In the case of oxytocin, this concerned coordination from the first social recognition and sexual interaction to mating, pregnancy, and care of the offspring. Vasopressin was found to be linked to agonistic behavior, in defending a territory.

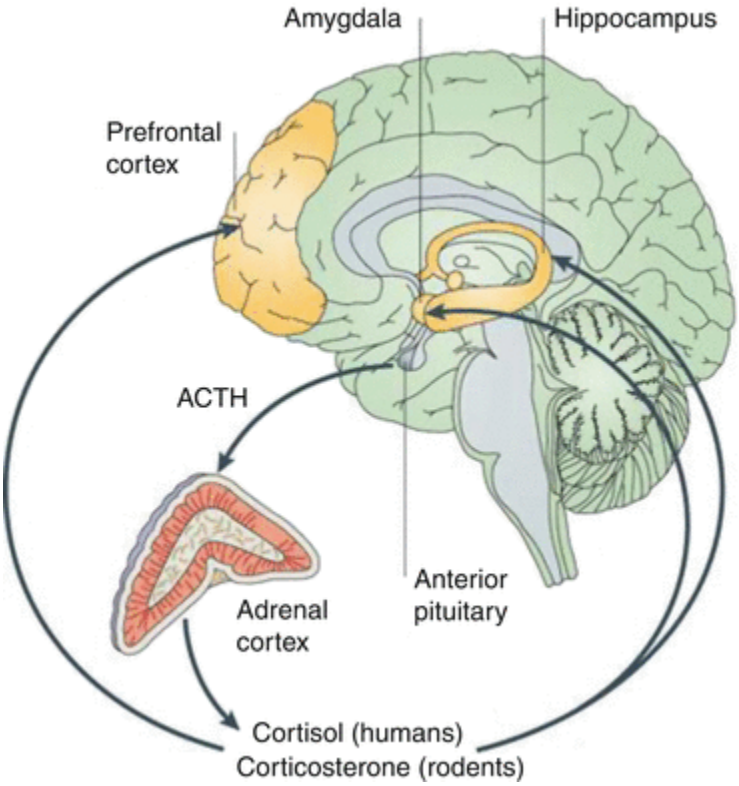


Fig. 64.2 HPA axis. Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis. The scheme demonstrates parts of the limbic system, that is, amygdala, hippocampus, prefrontal cortex, the anterior pituitary, and the adrenal cortex in the context of the intrinsic feedback connectivity (From Krugers et al. (2010 Oct). Nat Rev Neurosci 11(10):675-681, used with permission from the Nature Publishing Group)

All of these peptides participate in the stress response. In today's view, physical stressors convey via ascending aminergic pathways excitatory information toward the PVN. Psychological stressors are processed in the limbic brain structures and trans-synaptically modulate PVN function to secrete the CRH neuropeptide cocktail, which drives the neuroendocrine HPA axis, the sympathetic nervous system, and the behavioral response to the stressor. In the limbic circuitry, the amygdala does process stressful information into emotions driving the PVN, while in hippocampus these emotions are labeled in time, space, and context for storage in the memory. Pathways from hippocampus and prefrontal cortex communicate with amygdala and PVN. Limbic regions involved in cognitive processes and emotional responses are targets for modulatory influences of CRH, oxytocin, and vasopressin and other neuropeptides during behavioral and physiological responses to stress. Finally, the glucocorticoids secreted from the adrenals also target the limbic system, particularly those circuits that initially triggered the psychological stress reaction. The glucocorticoids exert very potent actions in concert with the other signals to modulate ongoing activity with the goal to facilitate behavioral adaptation.

> Quote 1: Glucocorticoids target the limbic circuitry where emotions are burned into memory and labeled in time, space, and context for appropriate retrieval at a later moment. The autonomic and HPA axis activity reflect the output of limbic processing of stressful information.

These processes induced by stress are superimposed on the basal activity of the HPA hormones.
B and F are secreted under basal conditions in hourly pulses. The hormones are produced within minutes, the pulse usually lasts about 20 min followed by a quiescent period until the next pulse arrives in about an hour later. The ultradian rhythm occurs in humans, monkeys, and other mammals including rodents. The pulses are thought to synchronize and coordinate daily activities and sleep-related events. Studies using automatic frequent blood sampling showed that B pulses are increased in amplitude toward the activity period, a pattern reflected in the free hormone changes measured in the extracellular fluid in brain using microdialysis (Fig. 64.3).

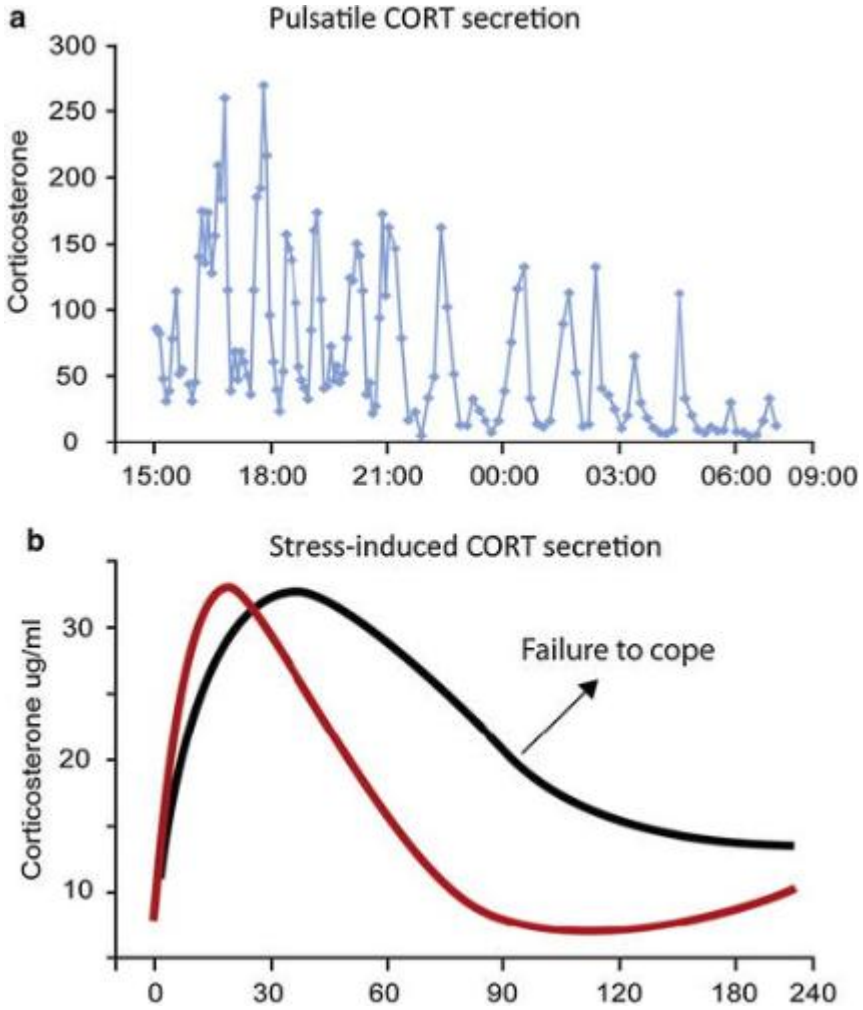


Fig. 64.3 Stress response and ultradian B rhythm. Pulsatile (a) and stress-induced CORT (b) secretion. The latter figure shows that a prolonged secretion of CORT develops under conditions of failure to cope with stress

The hypercortisolemia, during severe depression, is a result of the increased amplitudes of both, ACTH and B pulses, particularly at the nadir of the circadian rhythm. This finding would explain the flattening of the overall circadian rhythm in ultradian pulses characteristic for depression; the pulses have a larger amplitude during depression particularly at nadir.
Inflammatory disorders are characterized by increased frequency rather than amplitude of the ultradian rhythm. During aging, the ACTH-B pulsatile pattern becomes disordered as is reflected in the loss of circadian changes in daily activity and sleep-related events. The pattern of pulsatility therefore varies over physiological and pathological conditions. Accordingly, frequency encoding is an important modus operandi of the HPA axis.
The pulse pattern appears crucial for the responsivity to stressors. In a recent study, we have modeled ultradian variations in stress responsiveness by artificially creating different patterns of B in adrenalectomized (ADX) rats. The pulsatile administration of B facilitated a brisker neuroendocrine response to stress, which was markedly greater in the rising than in the falling phase of a B pulse. This differential phase-dependent effect was also seen in emotional reactivity and the behavioral response to noise, which was much greater in the rising phase. The finding raises the possibility that stress responsivity may show hourly changes, a notion that has not been investigated yet.
The question then is how responsiveness of glucocorticoid target genes in hippocampus may change under different regimes of pulsatility. This question was examined by comparing the expression of the GR and its target genes Gilz and Sgk-1 to patterns of B. Rats were implanted subcutaneously (sc) with vehicle or 40% B pellets known to flatten ultradian and circadian rhythmicity while maintaining daily average levels, or with 100% B pellets mimicking pathologically high B levels. The findings showed that the stable (non-pulsatile) concentration of circulating B released from the sc pellets dose-dependently downregulated GR and attenuated GR nuclear translocation in response to an acute B challenge, a finding that was reflected also in attenuated expression of Gilz and Sgk-1. The data suggest that sustained stable B levels

that disturb pulsatility can cause resistance to an acute challenge of GR signaling and target gene responsiveness (Fig. 64.4).

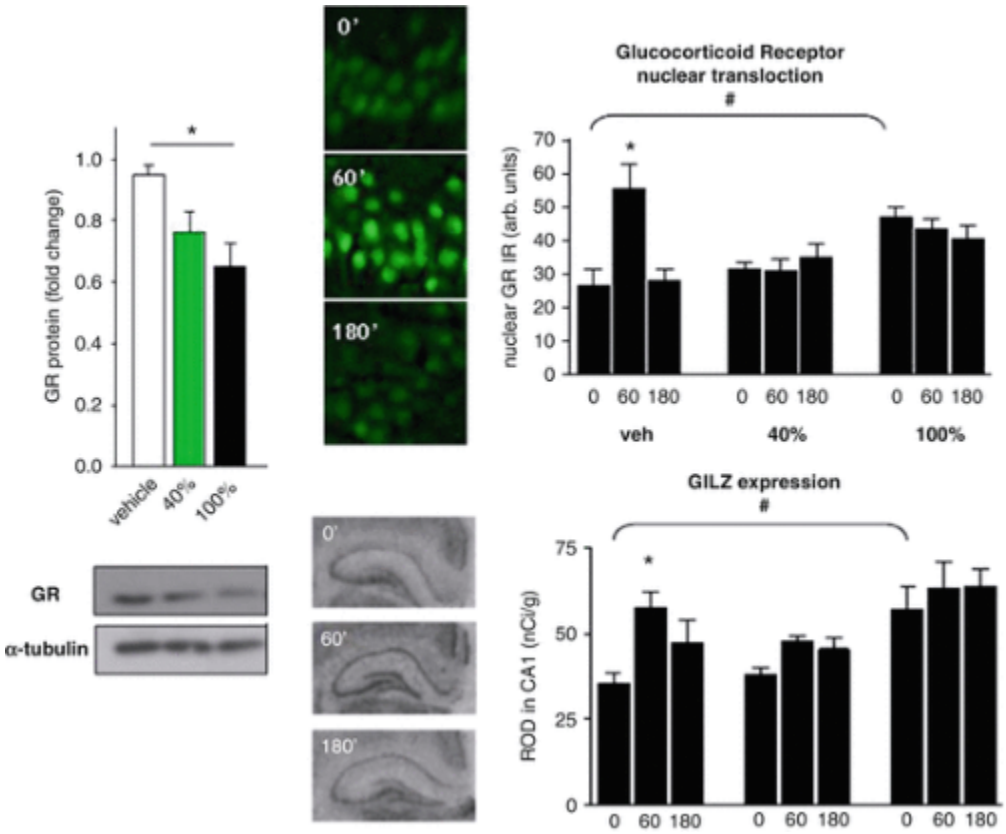


Fig. 64.4 Ultradian B and target gene responsiveness. Measurement of GR translocation to the nucleus and Gilz expression in hippocampus in response to a B pulse in animals with stable circulating B levels because of a B pellet implant versus sham implanted animals. Note that only in the vehicle animals GR translocates and Gilz responds (Adapted from Sarabdjitsingh et al. (2010 Mar). Endocrinology 151(3):1177-1186)

Actually, the reverse is achieved if an individual is exposed to chronic stress even though administration of exogenous B is often suggested to mimic the chronic stress condition. Chronic stress represents Selye's resistance phase or McEwens allostatic load condition. This is also a time of lability in homeostatic regulation which is characterized by enhanced responsiveness of brain substrates to acute challenges and exposure to B and F. This enhanced B responsiveness in animal models of chronic stress has been exploited extensively in cellular and molecular studies to identify "plasticity genes," that is, genes that depending on context convey either a positive or a negative outcome in physiological regulations and behavior. Interestingly, psychotic and depressive states are also characterized by homeostatic instability, with large ultradian swings in circulating F, particularly at the nadir, resulting in a flattened circadian rhythmicity. In depressives, the stress response results in prolonged glucocorticoid secretion implying resistance to the feedback action of glucocorticoids in the face of central hyperdrive. This has been exploited in the well-known dexamethasone (Dex) suppression test used to identify abnormalities in HPA axis functioning. In this test, HPA axis activity escapes Dex suppression because the central drive toward the axis overrides the suppression exerted by Dex at the pituitary level.

> Quote 2: For medical science it is worth to examine how pulsatile glucocorticoid therapy might shape the right hormonal conditions for resilience and mental health.

## The Essentials of Glucocorticoid Action

The actions exerted by glucocorticoids in the brain on processing of information underlying psychological stress reactions follow the guiding principle in any stress reaction as was explained in detail by Allan Munck (1984) and previously by Marius Tausk (1952). B and F feed back precisely on those processes that initially activated the HPA axis. This can be an inflammatory response, a metabolic disturbance, a reduction in blood volume or -as is the topic in this chapter - a

neurochemical reaction to a psychological stressor. These initial reactions are essential defense mechanisms, but may become themselves damaging if they overshoot. Glucocorticoids prevent these initial reactions from overshooting or as Marius Tausk said metaphorically: "glucocorticoids limit the water damage caused by the fire brigade." Exogenous glucocorticoids are indicated where the endogenous hormone is insufficient to contain inflammatory or immune disorders.

Over the past decades our research led to a conceptual framework explaining how the HPA axis and glucocorticoid hormones, in concert with catecholamines released after activation of the sympathetic nervous system and neuropeptides, can coordinate functions underlying the initial stress reactions with the management of later adaptations. It appeared that the very same glucocorticoids B or F first rapidly promote stress reactions and then contain these initial stress reactions providing the energy to cope and to recover, while promoting behavioral adaptation (see also Fig. 64.7). This concept combines the initial thoughts of Selye and Ingle, pointing out that glucocorticoids are equivalent to the stress response in their regulatory and permissive actions, merging them with the viewpoint of Munck and Tausk that glucocorticoids actually contain these initial stress reactions. Moreover, our concept is built on data showing that the enhancing and attenuating actions exerted in a temporal fashion by one single glucocorticoid hormone are mediated by the complementary functions of MR and GR in the limbic brain.

> Quote 3: Glucocorticoids act as a double edged sword in coordination of brain and behavior. The hormone can enhance the pulse and the stress reactions, which it then subsequently suppresses while promoting recovery, behavioural adaptation and memory storage of the experience for future use.

## Five Highlights

### Highlight 1: The Dexamethasone Story: How a Student Project Evolves in a Scientific Career

My (ERdK) contribution to neuroendocrinology started on December 1, 1968 as a PhD student at the pharmaceutical company Organon, with the task to explore the action of the synthetic glucocorticoid Dex in the brain. At that time Bruce McEwen had just described that a tracer amount (0.5 μg) of the naturally occurring glucocorticoid B ($^3$H-B), if administered to the ADX rat, was retained and accumulated in neurons of the hippocampus. We used tracer amounts of the potent synthetic glucocorticoid Dex ($^3$H-Dex). The low dose of 0.5 μg Dex was unfortunately poorly retained in limbic brain regions, which at that time was felt by me as a complete failure which would definitely jeopardize my scientific career. The poor retention of tracer Dex is a fact that was later confirmed, while working as a postdoc in Bruce McEwen's laboratory in the early 1970s. Dex was retained, though, in high amounts in the pituitary corticotrophs, a finding that established the gland as the principal site of action of the synthetic glucocorticoid in the suppression of stress-induced HPA axis activity. This pituitary preference of Dex also provided the mechanistic underpinning of the Dex suppression test which for several decades assisted diagnosis of aberrant HPA axis activity.

Only 30 years later we discovered why Dex is poorly retained in brain. This is because the synthetic glucocorticoids are recognized as an exogenous compound by the multidrug resistance P-glycoprotein (mdr1A Pgp) localized in the blood-brain barrier which exports the steroid. Pioneering research in the Netherlands Cancer Institute by Alfred Schinkel and Piet Borst had resulted in a mutant mouse with deleted mdr1A Pgp. If the tracer dose of Dex was given to these mdr1A (-/-) mutants, the steroid passed the blood-brain barrier, which in these mice was devoid of Pgp, and was retained in large amounts in hippocampal neurons. This finding suggests that Pgp indeed extrudes the synthetic steroid from brain. In subsequent studies focusing also on F, which does not naturally occur in mouse, it appeared that F is a substrate for Pgp explaining the reason why F is not retained in the hippocampus either. In the mdr1A knockout mice F was retained in amounts as high as B in hippocampal neurons.

Determination of the concentration of both steroids in extracts of human postmortem brain tissue using liquid chromatography mass spectrometry revealed that the ratio of B over F in the human brain was significantly increased relative to plasma. Thus, both in mouse and human brain the penetration of F is lower than that of B. This finding suggests a more prominent role for B in control of human brain function than hitherto recognized.

With this knowledge, the following scheme can be envisioned of Dex action on the HPA axis. The steroid blocks stress-induced HPA axis activity and therefore depletes the brain of B. Dex in low doses, however, poorly substitutes for the B-depleted brain because its brain penetration is hampered. Hence, the administration of moderate amounts of Dex would create a condition of "chemical-adrenalectomy" of the brain. We have tested this possibility and indeed found that under conditions that stress-induced ACTH and B release was suppressed by Dex, the CRH synthesis and release was not suppressed, a finding that supported the concept that low doses of Dex can create a hypocorticoid state of the brain.

Quote 4: Dex and F poorly penetrate (in rodents as well as humans) the blood brain barrier because of multidrug resistance P glycoprotein, while the entrance of B is not hampered. The story illustrates that a seminal observation may take 30 years to become understood.

Highlight 2: The MR:GR Balance Concept: A Product of Serendipity and Rational Research

Discovery

Because of the differential binding of B and Dex we suspected two types of receptors for the glucocorticoids in 1975. In the mid-1980s we discovered with a team of students, including Dick Veldhuis and Hans Reul, that indeed endogenous B binds to two nuclear receptor types: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), with a tenfold higher affinity to the former (Fig. 64.5, see for action mechanism Fig. 64.6).

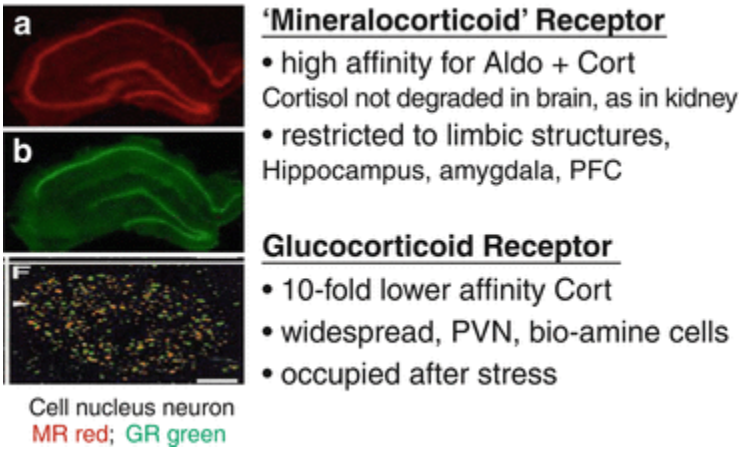


Fig. 64.5 MR and GR in the hippocampus. Co-localization of MR and GR in the hippocampus. The properties and localization of MR and GR are also described

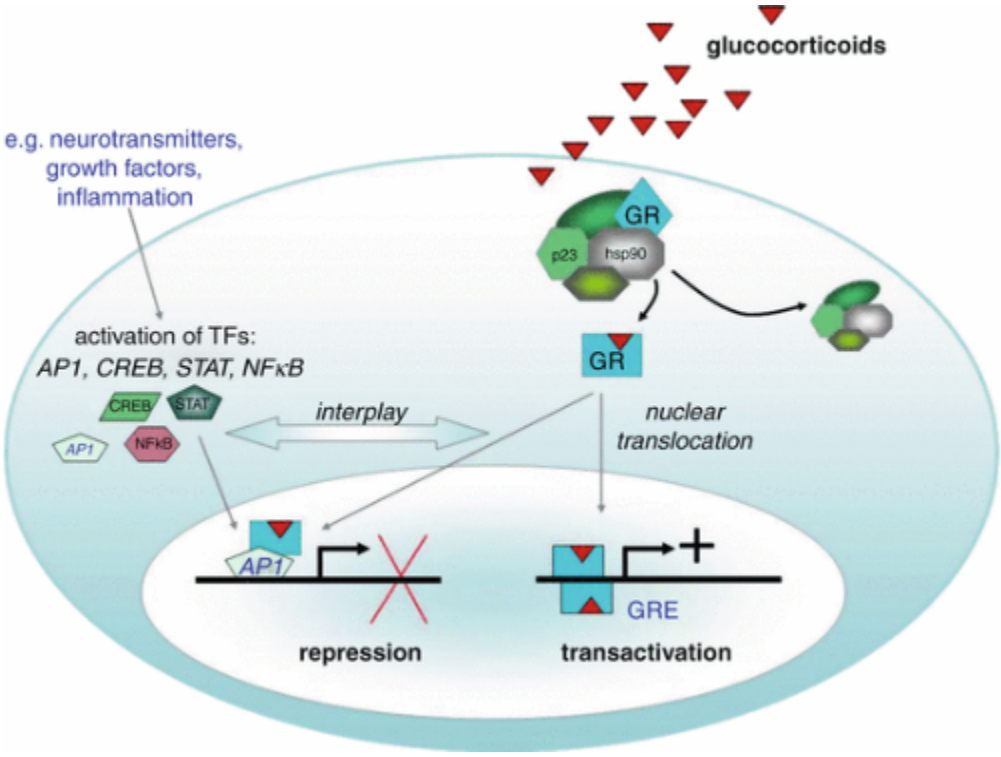


Fig. 64.6 Action mechanism of corticosteroids. Mechanism of glucocorticoid action. The receptors as part of a multimeric protein bind the glucocorticoids, which then dissociate and translocate to the cell nucleus. MR mineralocorticoid receptors, GR glucocorticoid receptors. MR and GR are transcription factors that regulate as dimers gene transcription; receptor function is also regulated by a cocktail of co-regulators. GR also interacts as a monomer with transcription factors (CREB, AP1, NFκB). MR and GR also function as membrane receptors modulating neurotransmission directly Courtesy of dr N Datson

Curiously, this MR was initially named (erroneously) the classical GR. This is because the tracer amounts of naturally occurring glucocorticoid B we used at the time were too low to detect the lower-affinity GR, but rather bound to MR. Only after cloning of the receptors by Ron Evans and the availability of specific antibodies the distinct localization and properties of brain MR and GR became apparent. I remember the "discovery" today as vividly as more than 25 years ago. I realized to have mined gold: one single stress hormone binding to two compelmentary receptor systems to account for basal and stressful regulations. The key paper by Reul and de Kloet, Endocrinology 1985 is still highly cited.
Another curiosity is that the MR is aldosterone selective in the epithelial cells in kidney, bladder, and sweat glands because of 11β-hydroxysteroid dehydrogenase type 2 that converts B into the inactive 11-dehydro-B congener. In brain, only the circumventricular organs are aldosterone selective in their regulation of salt appetite adding to the homeostatic role of the aldosterone selective MR in the maintenance of Na/K balance. In the rest of the brain only the type 1 isoform of this enzyme is expressed, which operates as a reductase and rather regenerates bioactive B from its inactive metabolite. MR in brain is promiscuous: the receptor binds aldosterone, progesterone, deoxycorticosterone, but sees predominantly B which circulates in excess.

> Quote 5: The co-localization and properties of MR and GR in limbic neurons can be exploited for study of steroid and stress effects in the brain.

Hypothesis

The MR : GR balance hypothesis predicts that, upon imbalance of these receptor functions, threats to homeostasis are less well communicated and coordinated among the various glucocorticoid targets. At a certain threshold this may lead to a condition of neuroendocrine dysregulation and impaired behavioral adaptation, which potentially can aggravate stress-related deterioration and promote susceptibility to stress-related disease for which the individual is genetically predisposed (Fig. 64.7a, b).

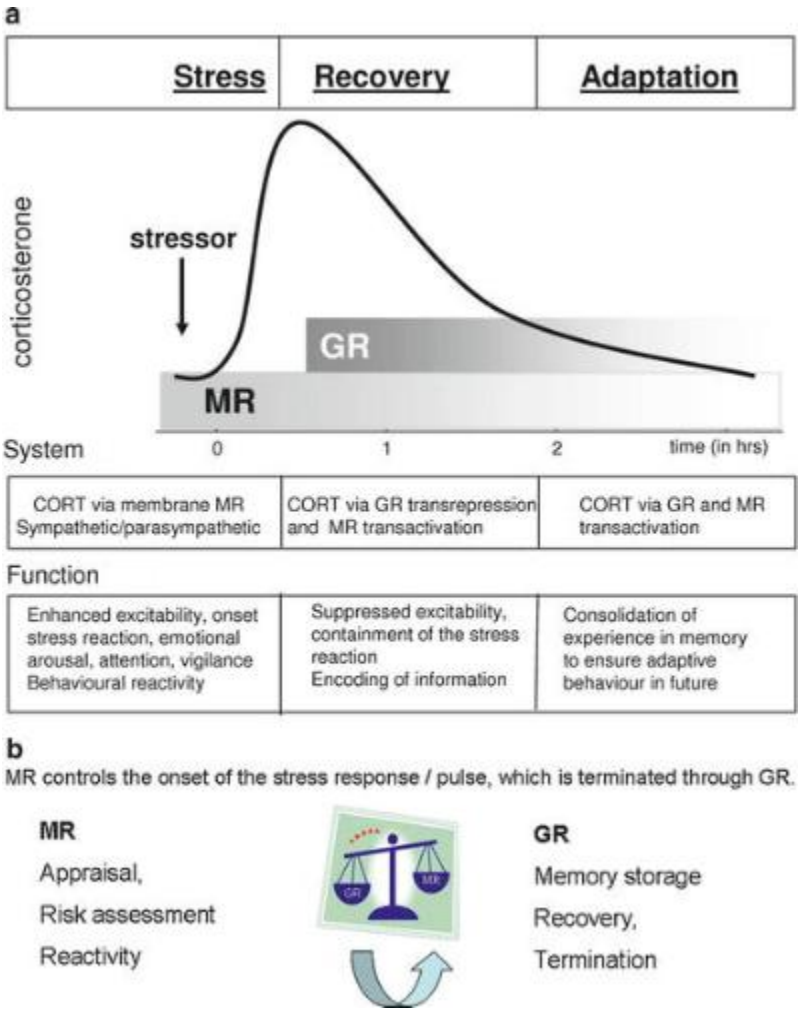


Fig. 64.7 (a) MR:GR balance hypothesis. Coordinate MR and GR mediated effects of CORT. The graph shows the phases of stress (the initial stress reaction) recovery and adaptation, during which the listed molecular, cellular, and behavioral effects, exerted by CORT, occurs (Reprinted with permission from Oitzl et al. (2010 May). Neurosci Biobehav Rev 34(6):853-866). (b) MR:GR balance hypothesis

The approaches to test this hypothesis are based on removal of the adrenals and subsequent replacement with B in dosages matching the affinity and specificity of each receptor, or the local administration of selective agonists and antagonists, site-specific inducible genetic deletion, or knockdown of either MR and/or GR. These studies showed effects mediated by both receptor types that were often complementary in nature, timing, and direction.

Neuroendocrinology

To illustrate this, we present the neuroendocrine effects of MR and GR. Thus, loss of function of MR in the hippocampus enhances basal pulsatility and stress-induced HPA axis activity. This effect slowly disappears over a few days upon chronic blockade of the MR and eventually results in another set point characterized by larger adrenals (ACTH stimulates mitosis) that now are more sensitive to ACTH. Blood pressure responses to stress were shown to be reduced by the MR antagonist icv, and this effect disappeared after denervation of the kidney suggesting, in addition to neuroendocrine regulation, a role of MR in autonomic outflow controlling volume regulation.
Conversely, GR in the PVN and pituitary mediates as expected the direct negative feedback action on stress-induced HPA axis activity, which is disinhibited by GR blockade. The GR in limbic structures mediates effects opposite to those via MR on neuroendocrine regulation and autonomic outflow. Superimposed on this, complementary MR- and GR-mediated actions on behavioral adaptation convey trans-synaptic signals modulating the stress response.

Behavior: Maze Studies

In the behavioral studies, the fundamental observation was made in a study by Melly Oitzl in the early 1990s using the well-known Morris water maze. The study focused on the role of the adrenals in Morris maze behavior. Performance was measured at several times after ADX, adrenomedullectomy, and administration of MR and GR antagonists icv. Administration of the glucocorticoid antagonist mifepristone up to 2 h after learning, with the goal to prevent the action of stress-induced B, blocked the consolidation of the experience. In the retrieval test 24 h later, the rats had forgotten the task and had to learn it all over again (Fig. 64.8). Similar findings were made in genetically modified mice having the GR knocked out locally in the forebrain or in which dimerization of the GR was no longer possible.

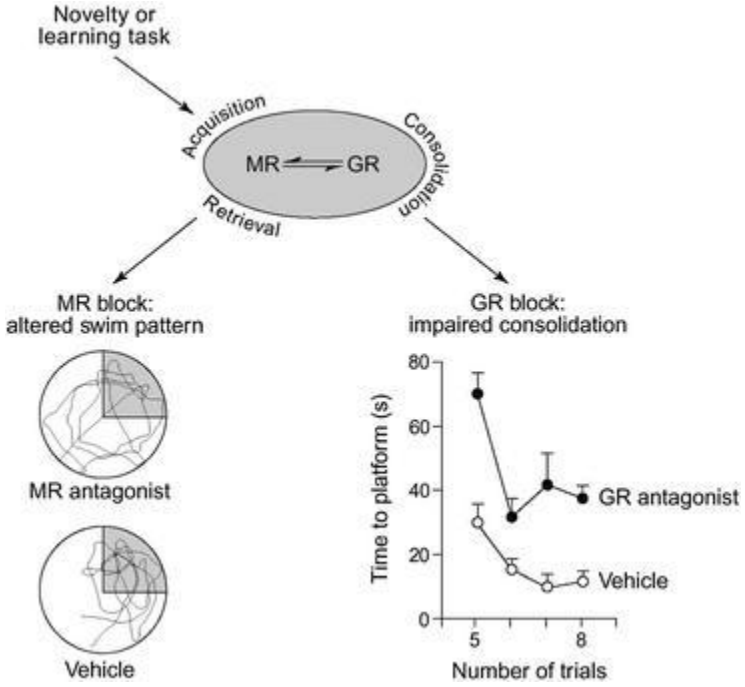


Fig. 64.8 MR and GR antagonists in the Morris Maze. Information processing modulated by CORT action via MR and GR in the Morris water maze. GR blockade with a glucocorticoid antagonist administered immediately after the learning session blocks consolidation of the learned swimming pattern measured 24 h later at the retrieval session. MR blockade only works immediately before the retrieval session and results in an altered search pattern for the platform (From de Kloet et al. 1999)

Blocking the MR with a mineralocorticoid antagonist after the learning trial was ineffective in affecting the storage of information. To achieve a blockade of MR function the antagonist had to be given either right before learning or briefly prior to the retrieval test. The latter effect was observed when the escape platform had been removed: the vehicle treated animals did show perseverance in behavior by remaining in the quadrant in which previously the platform had been located. However, blockade of the MR eliminated perseverance of the behavior: the animal started to search in the pool for alternative escape routes and thus apparently had switched its behavioral strategy. In subsequent studies administration of the MR antagonist prior to the learning trials rather than after also blocked the acquisition and hence encoding and consolidation of new information.

Stress and glucocorticoids are indeed capable of facilitating the switch between multiple memory systems in mice. In a series of studies by Oitzl et al., 2012 tests were designed to allow the mice to use either a caudate nucleus-based stimulus-response (habit learning) or a hippocampus-based spatial learning strategy. Naive mice used spatial strategies to locate an exit hole on a circular hole board at a fixed location flagged by a proximal stimulus, in this case a bottle. When the mice were either stressed or administered B before the task, 30-50% of the mice switched from a cognitive to a habit strategy. This switch between strategies was accompanied by a rescue of performance, while performance declined in the stressed mice that kept using the spatial strategy. Pretreatment with an MR antagonist prevented the switch toward the stimulus-response habit strategy, but did not rescue the deterioration of hippocampus-dependent performance. Similar findings were made in humans and further studies suggest that stress promotes habits at the expense of goal-directed performance. Oitzl and Schwabe's finding highlight that a coordinated MR- and GR-mediated action is involved in memory storage and retrieval of stressful learning experience.

As observed by Roozendaal, deQuervain, and McGaugh the glucocorticoid action in behavior facilitates a noradrenergic input in the limbic system. The timing of glucocorticoid manipulation is an important determinant; both hormones should be present at roughly the same time. However, pretreatment with glucocorticoids by an hour rather suppresses the emotional/noradrenergic effects on storage and retrieval. The importance of timing is supported by electrophysiological studies in the hippocampus and basolateral amygdala, showing synergy between the two hormones when applied simultaneously, but a suppressive action by corticosteroid when applied in advance of noradrenaline. Thus, only if the stress, noradrenergic, and glucocorticoid inputs are intrinsic to the learning experience the encoding and consolidation of information is enhanced.

> Quote 6: In the limbic brain MR is involved in appraisal of stressful information and the onset of the stress response. Via GR recovery from stress and information storage is promoted in preparation for the future.

### Gene Variants

Genetic variations have been identified in the MR and GR, as well as in proteins that determine their transcriptional activity (Fig. 64.9). Splice variants have been identified in the translated and untranslated regions of the receptors. Single nucleotide polymorphisms (SNPs) and haplotypes were found that lead to amino acid changes in the receptor proteins or - if present in the promoter regions - to differences in gene expression.

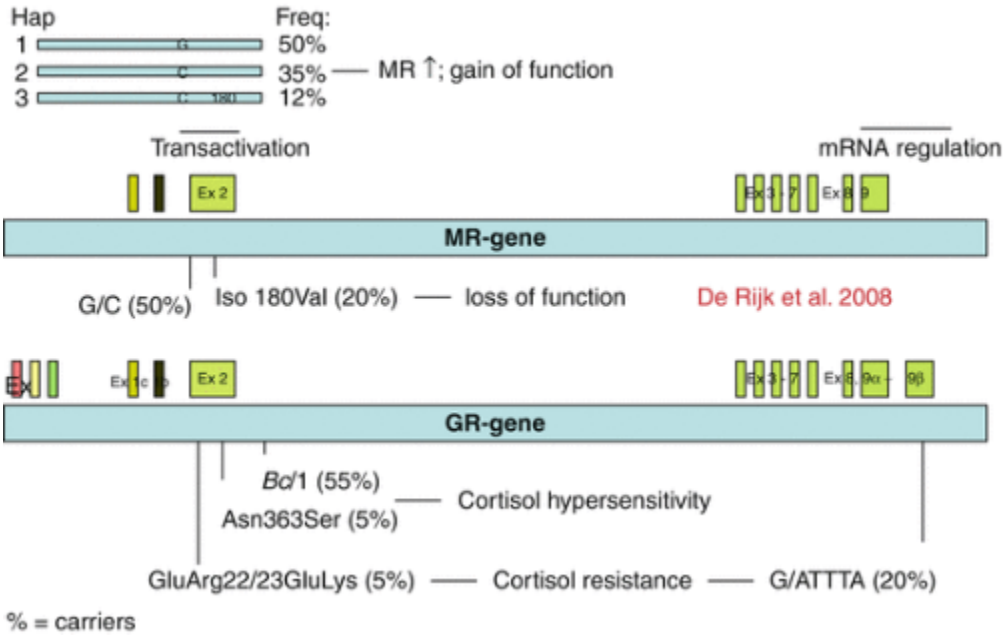


Fig. 64.9 Gene variants of MR and GR. An overview of the common genetic variants of the MR and GR and their effects on HPA axis reactivity (Based on data from Derijk et al. 2008)

In GR, the E22/E23 variant occurring as haplotype is associated with decreased glucocorticoid sensitivity, a more favorable metabolic profile and enhanced efficacy of antidepressants. In contrast, the N363S SNP demonstrates increased glucocorticoid sensitivity, high stress-induced F responses in man, an unfavorable metabolic profile, and vulnerability to psychopathology. The BclI site is associated with increased glucocorticoid sensitivity, unfavorable metabolic profile, and vulnerability to psychopathology. G/A TTTA located in the 3′ untranslated region stabilizes GR mRNA and is associated with high stress-induced F responses.

In the MR gene, Roel de Rijk discovered a highly interesting loss-of-function MR I180V variant, which is associated with increased stress-induced responsiveness of the HPA axis and autonomic reactivity as well as with feelings of depression. In subsequent studies, Roel de Rijk with his students van Leeuwen and Liane Klok identified common haplotypes based on the functional MR −2 G/C and I180V single nucleotide polymorphisms (SNPs; hap 1: -2 G/180I; hap 2: −2C/180I; hap 3: −2C/180V) that are in linkage disequilibrium (LD) with SNPs in the gene's promoter region; hap 4 was not found in the human genome. A promoter region of 4 kilobases containing haplotype 2 resulted in 1.4 or 2.2 times higher gene transcription after transfection in human neuroblastoma cells in comparison to haplotype 1 or 3, respectively (van

Leeuwen et al. 2010). Together with previous work, the data of Klok et al. 2011 show that haplotype 2 results in the highest gene transcription, translation, and transactivation of target genes. Genetic association analysis showed that haplotype 2 (freq. 0.36) was associated with heightened dispositional optimism ($p = 0.001$) in one study and with less hopelessness ($p < 0.05$) and rumination ($p < 0.001$) in a follow-up study. In both studies, effects were restricted to women.

> Quote 7: We propose that the common and functional MR haplotypes might relate to significant variability in MR expression in the brain, conferring inter-individual variability in susceptibility for psychopathology.

Highlight 3: The U-Shaped Response to Corticosterone (B): How It All Began

In 1990, I (MJ) tested a drug, developed by a pharmaceutical company and supposedly acting on serotonin-1A (5-HT1A) receptors. To assess the efficacy of the drug, I compared its action with that of 5-HT itself. After having sent the manuscript off to a journal, we received the comments of the reviewers and were asked to perform some extra experiments, which is rather typical. Meanwhile I had become interested in the effects of corticosteroid hormones in the brain. So, using my time efficiently I decided to do the extra experiments at the end of the day, after having finished my experiments on corticosteroids. And then the problems started: I was not able to reproduce the earlier findings with the drug and 5-HT, so that this limited series of extra experiments started to turn into a nightmare. I changed everything: pipette solutions, buffers, the settings of the pipette puller, etcetera. It actually took me more than a month to realize that the experiments with corticosteroids that I carried out every day until 4 pm greatly affected the responses to 5-HT which I tried to measure after 4 pm. Once that had dawned on me, it was only a small step to specifically investigate this interaction.

Activation of the 5-HT1A receptor in hippocampal CA1 cells increases the conductance of an inwardly rectifying K-channel, causing the membrane to hyperpolarize. It turned out that in the absence of B (i.e., in adrenalectomized rats), activation of 5-HT1A receptors causes a relatively large hyperpolarization. Selective activation of the MR is associated with very small responses to 5-HT. If GRs are activated in addition to MRs (e.g., as occurs after stress), this leads to a slow enhancement in 5-HT1A receptor-mediated responses. The latter was found to depend on DNA-binding of GR homodimers, underlining the genomic nature of this effect. Overall, 5-HT1A receptor-mediated responses therefore depend on the dose of B in a U-shaped fashion.

The U-shaped dose dependency seems to hold more generally for CA1 pyramidal cells. Thus, the influx of calcium through L-type voltage-dependent calcium channels, as well as properties that are linked to this calcium influx (e.g., firing frequency accommodation), show a highly comparable U-shaped dose dependency for B (see Fig. 64.10). It still has not been resolved whether this similarity can be explained by transcriptional regulation of a GR-target gene that subsequently modulates both 5-HT1A and L-type calcium channel function.

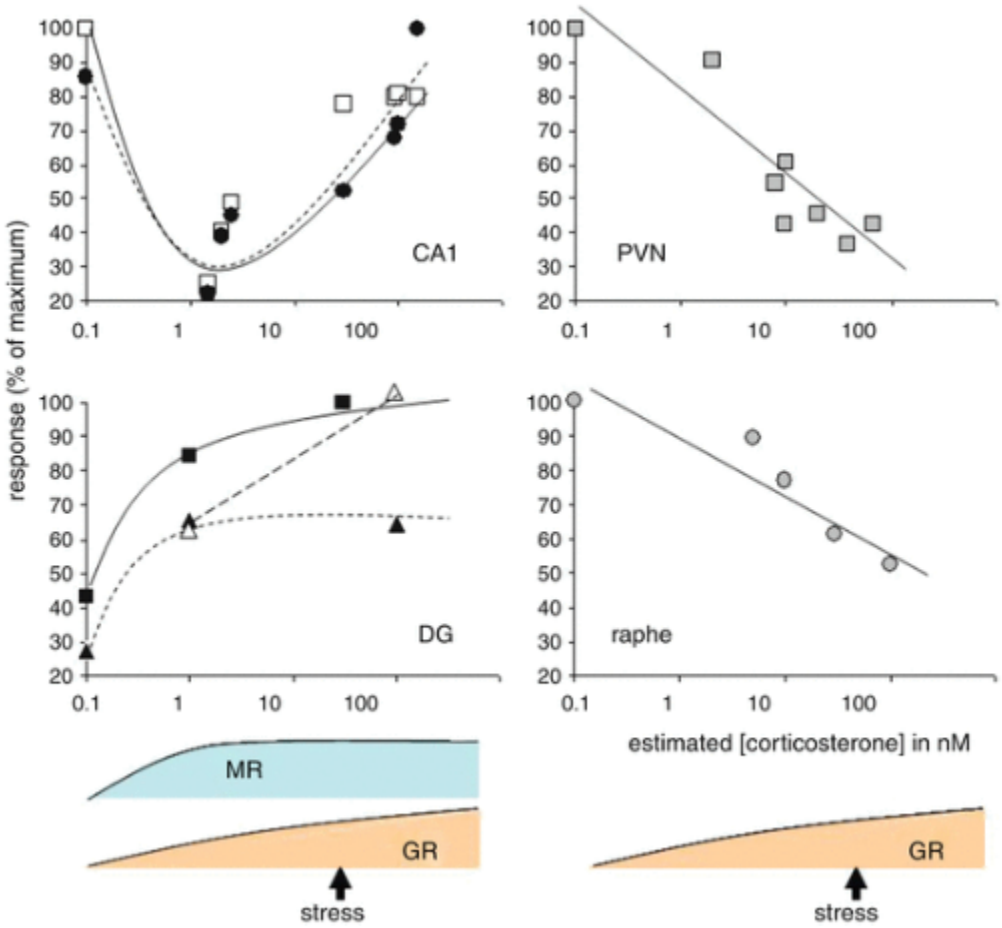


Fig. 64.10 U-shaped MR- and GR-mediated actions. Dose-response relationships for cellular effects of corticosterone in the CA1 hippocampal area, the dentate gyrus (DG), the paraventricular nucleus of the hypothalamus (PVN), and the dorsal raphe nucleus. The diagrams show hormone responses expressed as a percentage of the maximal response in various brain regions. The concentration of corticosterone is a rough estimate of the local concentration, based on the solutions perfused on in vitro preparations or derived from the plasma concentration when fluctuations in hormone levels were accomplished in vivo. In the CA1 area, both the amplitude of depolarization-induced calcium currents (open squares) and the hyperpolarization caused by serotonin-1A receptor activation (filled circles) display a clear U shaped dose dependency. The descending limb is linked to activation of MRs (see below), while the ascending limb is associated with gradual GR activation on top of already activated MRs, as occurs after stress. DG granule neurons show a clear effect on the field potential (filled squares) and single cell response (filled triangle) caused by activation of glutamatergic AMPA receptors; this effect is linked to MR activation. Although these cells also abundantly express GRs high doses of corticosterone do not give additional changes in the signal, except when tested in chronically stressed rats (open triangles). Neurons in the PVN and raphe nucleus primarily express GRs. In these cells a clear linear dose dependency can be seen for the frequency of spontaneous GABAa-receptor mediated synaptic events (gray squares) and the inhibition caused by serotonin-1A receptor activation (gray circles) respectively (Based on Joëls (2006 May), 27(5):244-250)

The U-shaped dose dependency, however, is not generally true for the various areas in the brain in which corticosteroid actions have been investigated. Obviously, brain cells that mostly express GRs but very low levels of MR (or vice versa, like CA3 hippocampal cells) will deviate from this U-shape dose dependency. In these cells, generally the dependence on the dose of B exhibits a linear shape. But even in cells that do express both receptor types for B, there is not always a U-shaped dose dependency. For instance, dentate gryus cells which express both MR and GR, appear to respond quite well to specific activation of MR but not of the GR. Administration of a high dose of B to dentate cells in vitro, - which supposedly activates GR - caused similar changes in gene expression of calcium channel subunits as seen in the CA1 area, but this was not translated to the protein level, nor did it cause an increase in calcium influx. This means that the response of specific brain cells to a range of B doses needs to be tested on a region-by-region base.

Quote 8: The response of the brain to a wave of B is a composite of the responses of cells in the various

parts of the brain carrying receptors and the way in which these areas are interconnected. These aspects need much more attention in future research.

Highlight 4: Metaplasticity of the Response to Corticosterone (B): Serendipity All Over Again

While corticosteroid actions in the brain have classically been considered to arise exclusively through transcriptional regulation of response genes, it has become evident over the past decade that the hormone also induces rapid effects which develop too fast as to involve gene transcription and translation. In 2005, we described that application of B to CA1 hippocampal cells quickly and transiently increases the frequency of miniature excitatory postsynaptic currents (mEPSC), each of which represents the postsynaptic response to the spontaneous release of one glutamate-containing vesicle. Follow-up experiments demonstrated that this increase is probably due to an enhanced release probability of the vesicles, rather than having more functional contacts. The rapid effect does not depend on protein synthesis and is accomplished with a conjugate of B and BSA which cannot pass the plasma membrane, indicating the involvement of a membrane-located receptor. In fact, this receptor seems to be located in the presynaptic membrane and linked to the ERK1/2 signaling pathway.

Significant effects were obtained with a B dose of 10 nM, which suggested involvement of GRs rather than MRs. It therefore came as somewhat of a surprise that both pharmacological experiments and studies in genetically modified animals supported involvement of MRs rather than GRs. The fact that these presumed membrane-MRs are activated with a tenfold lower dose of B than the intracellular MR suggests that this pool of receptors provide the means to quickly respond to shifts in corticosteroid level, such as may be expected after stress or during the ultradian pulses. This would lend an important role to the MR which hitherto was considered as the Cinderella of the corticosteroid receptors, being substantially occupied already under rest, hence leaving a very small dynamic range under conditions of stress.

Since stressful conditions not only involve the hippocampus but nearly always also cells in the basolateral amygdala (BLA), we were curious to see if principal cells in the BLA also exhibited these fast MR-dependent responses in glutamate transmission. This turned out to be partly true. Thus, BLA cells also show an increase in the mEPSC frequency; however, the onset of the response was slightly more gradual and, most importantly, the frequency did not return to baseline upon washout of the hormone but rather stayed high.

This was an interesting observation, though not exactly earth-shattering, but we decided to prepare a manuscript to report these findings. To give the finding more "body" we wanted to perform some pharmacological studies, to prove that the enhanced mEPSP frequency in the BLA was caused by MRs, like in the hippocampus. That turned out to be easier said than done: We could not reproduce the earlier observed B-induced increase in mEPSC frequency in the BLA. If the experiments had been performed by a starting PhD student or postdoc, I might have considered that he or she performed the experiments wrongly. But coming from the most experienced patch-clamper in town (Henk Karst), the data had to be true and we had to look for another explanation. We thought of all possible explanations (different animal supplier, different chow, different chemicals to make the buffer, you name it), but the only thing that had changed over the past months was the fact that we had a new animal caretaker in our animal facility. While the previous person was rather subdued and ready for retirement, the new man was extremely energetic and every morning came whistling into the animal facility, bustling with buckets and lavishly spreading soap over the floor. The animals, used to the quiet atmosphere that up till then had reigned in the animal house, were probably scared out of their wits. We considered the possibility that they suffered from "novelty" stress and decided to address this situation by dedicated experiments. And sure enough, if we stressed the mice before preparing the slices, B application in vitro resulted in a decrease in mEPSC frequency (Fig. 64.11). This decrease (as opposed to the increase) appeared to depend on non-genomic actions via a GR, involving the endocannabinoid receptor 1. Thus, the rapid response of BLA neurons to B depends on the recent stress history of the animal, a phenomenon that we dubbed "the metaplasticity of the response to stress." Thanks to the new animal caretaker! It subsequently turned out that the shift from enhancement to decrease in mEPSC frequency critically depends on the sustained nature of the initial rapid effect. This lengthy effect is gene-mediated and involves both MR- and GR-dependent steps.

Quote 9: The response of brain cells to B is not necessarily always the same. The recent history of the organism may greatly affect the final outcome, as demonstrated in the BLA. To what extent this principle also holds for other brain areas requires more investigation.

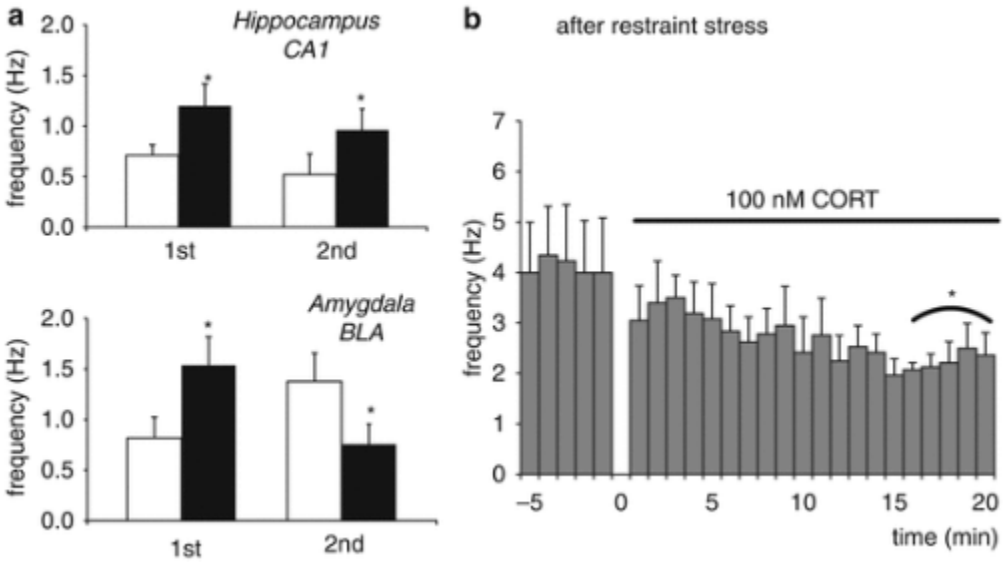


Fig. 64.11 Metaplasticity in the Basolateral Amygdala. In the presence of corticosterone (black bars in a) mEPSC frequency in the hippocampus is increased. A similar response is seen after a second pulse of corticosterone. In the BLA, however, the first pulse lastingly increases mEPSC frequency, which is quickly reset by a second pulse. Also after restraint stress (b), corticosterone suppresses mEPSC frequency, * means significantly different from baseline (Based on Karst et al. (2010 Aug 10), 107(32):14449-14454)

Highlight 5: Nothing Is Written in Stone: SHRP, Early Adversity, and Programming the Brain

As early as in the 1957, Seymour Levine observed that handling of newborn laboratory rodents during the first 2 weeks of life resulted in an adult phenotype characterized by reduced emotional and stress reactivity. This seminal observation has since then been reproduced in numerous laboratories. In retrospect, the comparison between handled and non handled animals represents one of the most robust examples of phenotypic plasticity induced by early life experience which has permanent consequences for emotional reactivity and cognitive performance in later life. The underlying mechanism is now better understood since it appeared that such early life experiences can modify (permanently) the expression of genes by DNA methylation of promoter regions. Here, we summarize two concepts: (1) stress hyporesponsive period (SHRP): the effect of early experience, (2) later life outcome: three-hit hypothesis and the mismatch concept.

SHRP: The Effect of Early Adversity

During the first 2 weeks of the life of rodents, the adrenal B secretion shows little responsiveness to stressful stimuli which otherwise in the adult trigger a large response. The most proximal cause of the SHRP is hyporesponsiveness of the adrenals, as is revealed by exogenous ACTH injections. Separation of the dam from the pups causes a slow activation of B secretion, taking several hours to develop. At the end of the deprivation period, the pups' HPA axis response to a mild stressor is enhanced and the SHRP has become disrupted. If during the 24 h period maternally deprived pups were stroked every 8 h for 45 s with a warm wet painter's brush, the stress-induced activation of cfos and CRHmRNA in the PVN and pituitary ACTH release were abolished. Intriguingly, mimicking the sensory stimulation by the mother did not affect stress-induced B secretion in the deprived newborns unless the pups were also fed.
The slow rise in B that occurs during the first separation from the dam does not occur the next day as if the pup has learned to predict the return of the dam. However, in spite of this rapid habituation to repeated maternal absence, the pup's HPA axis continues to show enhanced responsiveness to novelty. In the pioneering studies of Regina Sullivan on odor-learning, the odor system is fully developed and functional the first week of life. At approximately pnd 10, pups exhibit preference to novel odors, even if they are paired with negative stimuli, by co-activation of the locus coeruleus - olfactory bulb pathway. Then, odor-avoidance behavior appears and is associated with the activation of neural processes in amygdala and piriform cortex. Interestingly, during the SHRP, when the dam is away, the odor aversion neuronal system can be activated prematurely and aversive memories can be formed as long as the B levels are elevated in blood and amygdala. Such elevated B levels are attained during the first long-term absence of the dam. Nikos Daskalakis and I

found that priming of the amygdala fear pathway only occurred if during prolonged maternal separation the pups were additionally isolated in a novel environment.

A great variety of paradigms are used to study the effect of early experience. These include the effect of infections interfering with maternal care, single long term-maternal deprivations, or repeated maternal absence. It has become apparent that these conditions need to be standardized. Since the pups rapidly adapt to repeated maternal absence, their housing conditions during absence of the dam are an important determinant for outcome. Moreover, besides the actual absence of the dam, also the care the pups receive upon reunion is an important experience-related factor. Hence, variations in maternal care have been used as model to study later life outcome by comparing the extremes: low and high maternal care groups.

### Later Life Outcome: Three-Hit Hypothesis and the Mismatch Concept

Adult rats that had received as pups low maternal care showed enhanced emotional and stress reactivity together with impaired cognitive performance. This phenomenon is well established in the Long Evans strain, but not in other strains suggesting interaction with genetic background. To examine this aspect specifically, a rat line genetically selected for profound responsiveness of the dopaminergic system to apomorphin (the so-called apo-sus rat line developed, thanks to Alexander Cools in Nijmegen) was examined for the maternal care they received from the dam. Nikos Daskalakis discovered that adult apo-sus rats having experienced as pups poor maternal care develop a baseline prepulse inhibition deficit (PPI) Additional isolation rearing at weaning entirely abolished baseline PPI in the low maternal care apo-sus offspring and impaired their short-term memory. Although the stress-induced B secretion and prolactin release are enhanced, the dramatically enhanced brain response to an emotional stressor is particularly striking; this response seems to be only to a limited extent restrained by B. The data support the three-hit hypothesis of psychopathology: early life adversity enhances vulnerability of genetically predisposed individuals to a psychosocial stressor experienced during adolescence, resulting in a severe schizophrenia-like phenotype given the abolishment of PPI, a marker for sensorimotor gating, the impaired working memory, and the defect in social interaction, which are characteristic for patients suffering from schizophrenia.

However, recent findings have pointed out that the outcome of early life adversity actually depends on the later environmental context, suggesting that the above scenario pictured in the three hit hypothesis not inexorably and inevitably leads to disease and misery. "Nothing is written in stone" Gig Levine once noted when referring to the amazing plasticity of the brain. Indeed, it was found that early life experience can program through an epigenetic mechanism the activity of, for example, corticosteroid receptor genes as a special class of plasticity genes. A striking example is the phenotype of the rat that was deprived as pup for 24 h from maternal care. Melly Oitzl observed that during midlife these rats have become very susceptible to stressors which then activate an individual-specific trajectory of aging, which is reflected in their cognitive performance at senescence. While the control animals show a normal distribution during senescence, with most rats having mild deficits, some performing very well and others poorly, the deprived rats lack a large middle group: only those animals remain that perform either very well or poorly. Hence, an early life experience not always produces poor performance. No, it activates a mechanism that makes these animals more susceptible, for better or for worse.

Hence, the more susceptible phenotype imposed by glucocorticoid programming points to the phenotypic/genetic plasticity which underlies the concept of "predictive adaptive response." This concept implies that early life conditions may prepare for the upcoming life, with the goal to "match" future environmental demands. This concept has led to the hypothesis that a "mismatch" between early and later life conditions can enhance vulnerability to disease. Evidence supporting the hypothesis comes from studies showing that malnutrition and stress experienced during pregnancy produced smaller offspring with a lower birth weight and altered metabolism. It is thought that this response of the fetus to the current "in utero" conditions represents a reliable prediction for the upcoming life conditions as a safeguard for evolutionary success. Rosana Sibug (2005) found that these "in utero" conditions may go as far back as the implantation conditions of the blastocyst. For instance, in a mouse model for in vitro fertilization (IVF), in vitro culture of pre-implantation embryo's until the blastocyst stage revealed psychomotor and emotional changes later in life. Hence, the predictive adaptive response has served to explain why a mismatch between malnutrition during early life and abundant resources in later life enhances not only the risk for cardiovascular disease, metabolic syndrome and diabetes, but also for brain disturbances.

In the area of brain and behavior, evidence has become available supporting the predictive adaptive response. In the experiments by Danielle Champagne and colleagues it was first demonstrated that rats which had received as pups lower

amounts of licking and grooming by the dam also displayed altered morphological and electrophysiological consequences, and reduced MR and GR expression in hippocampus during adulthood. The lower the maternal care the shorter the dendritic branches and fewer the spine densities in the hippocampal CA1 area, while long-term potentiation (LTP) was impaired (Fig. 64.12). However, if the low maternal care offspring was exposed to B, the LTP response improved dramatically, while under the same conditions the LTP response of the high maternal care offspring deteriorated. The results were paralleled at the behavioral level: the low maternal care offspring performed worse than high care offspring in a non-stressful object recognition learning paradigm but much better than their high care littermates in learning a contextual fear conditioning paradigm, a quite stressful behavioral test. Hence, this finding provides support for the predictive adaptation concept because of the excellent performance of the low maternal care offspring under the stressful conditions.

> Quote 10: The stress diathesis and 3-hit theory suggest that a combination of risk (plasticity) genes with early adversity and later stressful life events inevitably produces a phenotype vulnerable for mental disorders. An alternative view is that the outcome of Gene x Environment interactions prepares an individual in anticipation for life to come, the "mismatch" concept. In the latter concept the programming of an emotional/cognitive reactive phenotype by early adversity will render the individual better equipped to cope with demanding situations. Hence certain plasticity genes (e.g. MR and GR) are important determinants for life scenario's linked to either a 3-hit or a mismatch outcome which may lead in its extreme form to either an excellent or poor health.

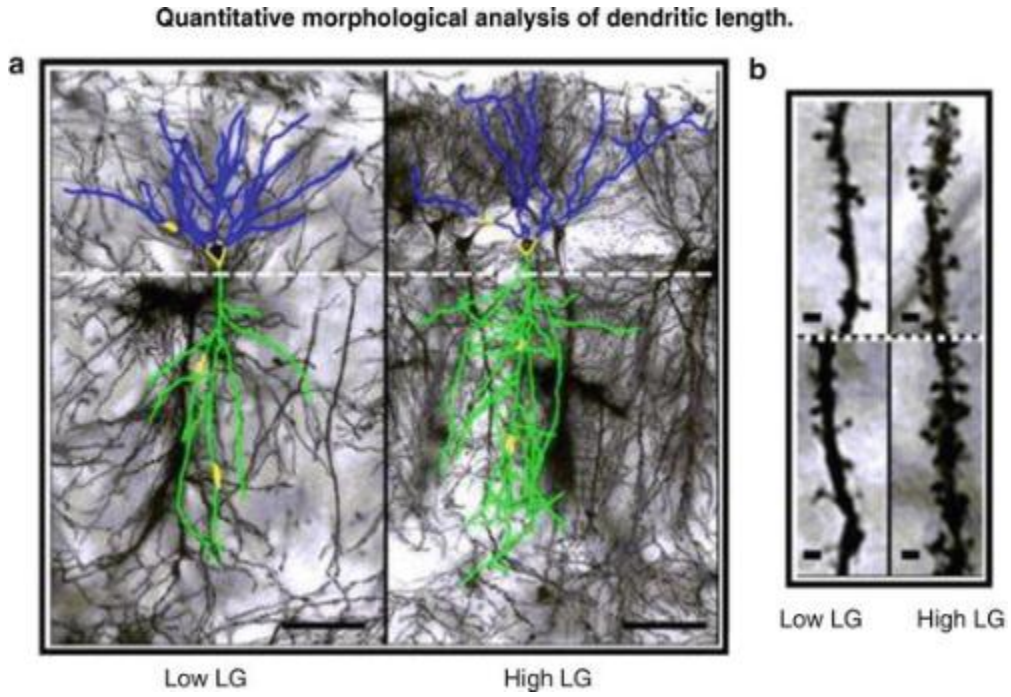


Fig. 64.12 Hippocampal CA1 morphology of low maternal care offspring. Effect of the quality and quantity of maternal care on dendrite structure of Golgi-stained hippocampal CA1 neurons of the 2 month- old offspring. LG, maternal licking and grooming (From Champagne et al. (2008), J Neurosci. 28(23):6037-6045)

# Outlook

## Concluding Remarks

Our contribution to Neuroscience in the twenty-first century is aimed toward forward statements given as quotes, which are based on fundamental concepts in stress and stress hormones. By doing so, we are fully aware that research findings usually sustain one generation of scientists lasting 20-30 years until the same question is addressed again but by others and with new techniques.

## Neuroendocrinology Remains Alive and Kicking

Admittedly, great discoveries in the field of hormones are quite some time ago. In the topic of stress, the Nobel prize in Physiology and Medicine went in 1950 jointly to Kendall, Hench, and Reichstein "for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects." And in 1977, to Guillemin and Schally "for their discoveries concerning the peptide hormone production of the brain" and the other half to Rosalyn Yalow "for the development of radioimmunoassays of peptide hormones." There have been 20 Nobel prizes in (Neuro)endocrinology, but the last one was 10 years ago, and neither Selye's "Stress" concept nor the Geoffrey Harris concept of neuroendocrine communication got awarded. Yet, Endocrinology has a big impact in the Biomedical Sciences. Why?
An interesting answer to this question can be best be given by a quote of Marius Tausk (1902-1980) on the occasion of the opening symposium of the Netherlands Endocrine Society, May 10, 1947. "Endocrinology is a concept, an approach, or even can be considered a method" Tausk said, "Whatever the specific endocrine subdiscipline, topic or subject might be, the binding element is the objective, which is the understanding how (hormonal) signals coordinate the processes in cells, tissues and organs."
Even though this statement was made more than 60 years ago, today it is as timely as ever. Most hormones are known, as is globally their mode of action. What remains one of the major challenges today is to discover how hormones manage to coordinate multiple and widely diverse molecular actions at the cellular level toward one integrated response of body, brain, and mind, resulting in behavioral adaptation.

## Mechanism

Genomics, proteomics, and metabolomics combined with genetic and imaging approaches are being used for identification of "plasticity genes" encoding stress-responsive pathways in the brain. Such plasticity genes are the basis of enhanced susceptibility to environmental and cognitive inputs and depend on experience-related factors. Such genes may preserve resilience and health, and protect against disease under beneficial conditions, but could alternatively enhance vulnerability in an adverse context. As pointed out by Belsky, an example of a plasticity gene is the 5HT transporter. The promoter region of this transporter can vary, with "short" and "long" repeats in a region: the 5-HTT-linked polymorphic region. The short form produces less of the 5HT transporter than the long form, and has been linked to enhanced vulnerability to stress and anxiety disorders. However, under favorable conditions the short form enhances resilience.
This viewpoint of differential susceptibility articulated by Jay Belsky proposes an alternative to the classical stress-diathesis concept which aims to identify individuals as particularly vulnerable to adversity because of their genetic makeup. The plasticity genes convey to individuals' enhanced susceptibility to early life experience, later life events, and environmental input, either resulting in enhanced vulnerability to disease during adversity, or resilience under beneficial conditions.
In fact, the stress response system, in particular B and F, are the key toward identifying these networks of plasticity genes since the very same hormone can change from protective toward damaging depending on the environmental conditions, and the MR:GR balance is thought to play a crucial role. The gene networks responding selectively to MR and/or GR stimulation, also under chronic stressful conditions, have been mapped and show an overrepresentation of genes involved in synaptic plasticity and morphogenesis (see the papers by N. Datson et al. between 2001 and 2011). One could call this unbiased approach in a mindset of extreme optimism the "endgame": the genome structure is known, now it is time to see how it works (Fig. 64.13). In our modest opinion, a real breakthrough in this timely business is only possible if this unprecedented new systems knowledge is combined with a smart experiment. Such an experiment could take into account that environmental input determines the brain's activity, which in turn provides the context to cells, governing the molecular changes. Glucocorticoids via its balanced MR:GR activities power this environmental input from brain to molecule.

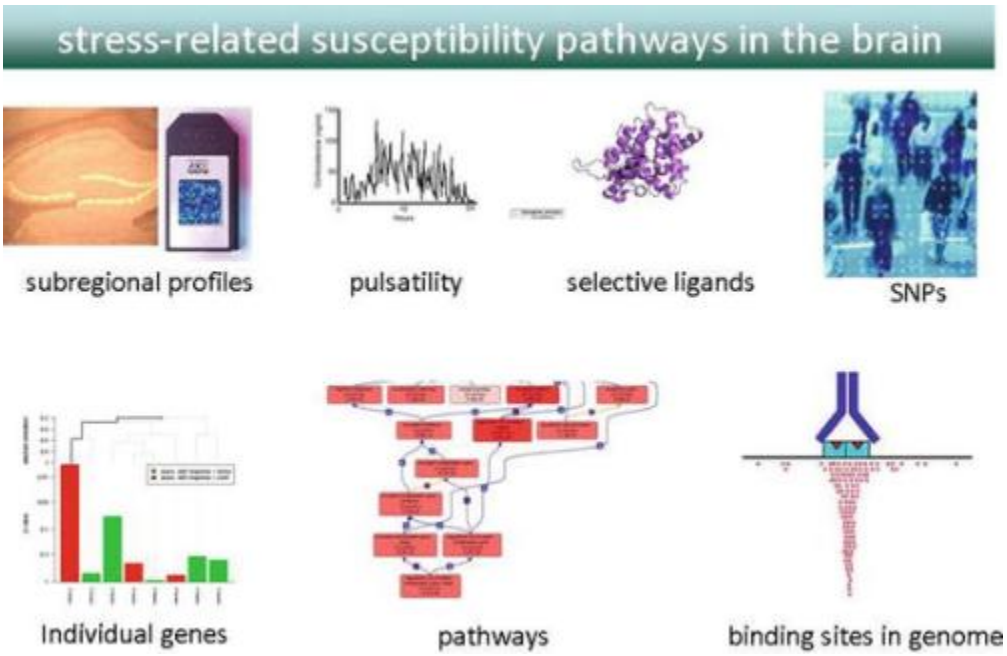


Fig. 64.13 The Endgame. Common methodologies in the coming decade to unravel to identify susceptibility pathways for stress hormone action in the brain (Courtesy of Nicole Datson)

## Integration

Glucocorticoid action is complex. For instance, the GR is encoded by only 1 gene, but the protein occurs according to John Cidlowski in dozens of variants. In addition, the receptor is surrounded in the cell by at least a few dozen proteins that in different patterns can enhance or suppress the activity of the receptor in the control of gene expression. Then, there are about 6,000 genes that appear responsive under different conditions, experiences, and behaviors to the environment via glucocorticoids. The hormone feeds back on that particular neural circuit that underlies processing of specific stressful information via complementary MR- and GR-mediated responses. This action exerted by glucocorticoids is coordinated with its actions on, for example, energy metabolism, plasticity, and other processes, also elsewhere in brain and body, in order to promote adaptation to the environment.

In the past 2 decades, quite some progress has been made in understanding how circulating corticosteroid hormones act in hippocampal circuits that harbor cognitive operations related to space and context. The new data have given more insight in the mechanism underlying the B-enhanced excitability mediated by membrane and nuclear MR on the one hand and the GR-mediated suppression of transiently raised excitability on the other. This cellular and circuit MR:GR balance translates into a still poorly understood process of coping with stress. Endocrine and behavioral responses were identified that matched specificity, nature, and duration as dictated by the properties of MR and GR at the cellular level. Gene variants can bias the MR:GR balance and early life events appear crucial for epigenetic modulation of MR:GR expression as well as the maturation of susceptibility pathways underlying coping with stressful information.

## Mental Health and Quality of Life

When using these approaches, the context and therefore the design of the experiment will learn how these responsive pathways can serve as mechanistic underpinning within a given cognitive and emotional mode of operation and how the stress - glucocorticoid switch can alter such operations from one mode into another. After all, a rapid onset in the HPA and behavioral response to stressors is the signature of a healthy resilient organism as long as coping and thus the termination of the response proceeds effectively. In other words, if an efficient transition of allostatic states occurs to achieve homeostasis. This knowledge eventually will lead to the identification of biomarkers that can be helpful in developing lifestyles and other measures required for design of a personalized medicine strategy that enhances resilience still present in the diseased brain, and that prevents rather than cures. Briefly, a strategy aimed to advance the twenty-first century Neurobiology of Mental Health.

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**Stress Research: Past, Present, and Future**

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