Stress Research

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Stress Research: Past, Present, and Future

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Contents

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 hypothalamus-pituitary-adrenal (HPA) axis. This action exerted by the glucocorticoids is mediated by a dual receptor system: mineralocorticoid (MR) and

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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.

Keywords

Adaptation – Ageing · Allostasis · Behavioral studies – Brain · Circadian rhythm – Cognition · Dexamethasone – Electrophysiology · Emotion · Gene variants · Glucocorticoids – Glucocorticoid receptors (GR) – Hippocampus = Hypercortisolemia – Hypocortisolemia · Hypothalamus-pituitary-adrenal (HPA) axis · Maze studies · Mechanism · Mental health and quality of life · Metaplasticity · Mineralocorticoid receptors (MR) · Mismatch concept · Neuroendocrinology · Neuropeptide – Neurotransmitters · Pro-opiomelanocortin (POMC) · Stress · Stress hyporesponsive period (SHRP) · Three-hit hypothesis of psychopathology · Ultradian rhythm

Abbreviations

5-HT	5-Hydroxytryptamine = serotonin
5-HTT	Serotonin transporter
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
LTP	Long-term potentiation
mdr	Multidrug resistance
mEPSC	Miniature excitatory postsynaptic current
MR	Mineralocorticoid receptor
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

Already in 1915, Cannon linked the sympathetic mediator adrenaline with the fight, flight, or fright response to cope with a threat, a notion that marks one of the first steps in the evolution of the stress concept (Table 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 latter term coined by Hans Selye in the same year to describe the "nonspecific reaction of the body to noxious stimuli" (Table 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; two major

Table 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 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 and Reul	Mineralocorticoid (MR) and glucocorticoid receptors (GR) in brain
1995	Karin	Glucocorticoid transrepression vs transactivation
2004	Meaney	Early life effect on glucocorticoid receptor methylation
2005	Tasker/Karst/Joels	MR and GR action at membrane

communication systems, the autonomic nervous system and the HPA axis, are extremely important in this respect.

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 stress 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 unfriendly 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 disorders 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, that is, adaptation to change. 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 to achieve this 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 increased vigilance, 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 before Wylie Vale identified corticotrophin-releasing hormone (CRH), which is synthesized in the paraventricular nucleus (PVN) of the hypothalamus. CRH synergizes with vasopressin in promoting the synthesis and release of ACTH, which is cleaved from the pro-opiomelanocortin (POMC) precursor and 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 as first demonstrated in a classical experiment by Dwight Ingle (1938).

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. 1). 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 concerns 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.

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,

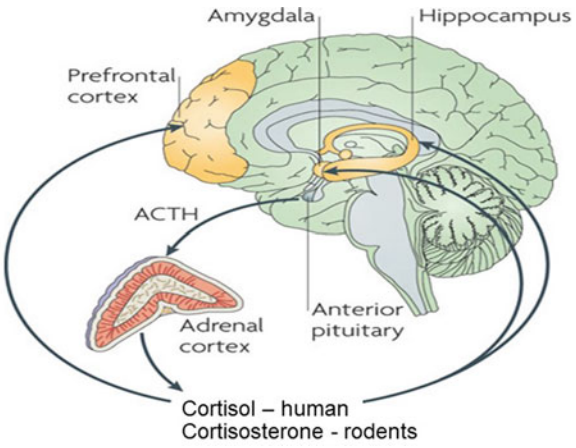

Fig. 1 HPA axis. Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis. The scheme demonstrates parts of the limbic system, that is, amygdala, hippocampus, prefrontal cortex, the anterior pituitary, and the adrenal cortex in the context of the intrinsic feedback connectivity. (From Krugers et al. (2010), used with permission from the Nature Publishing Group)

while in the hippocampus these emotions are labeled in time, space, and context for storage in the memory. The mPFC is exerting control by appraising controllability and selecting the stress-coping style with the goal to direct a *top-down* executive network to terminate the stress response as part of a behavioral adaptation program. Glucocorticoids secreted from the adrenals target in *bottom-up* fashion the limbic-prefrontal circuitry, particularly those circuits that initially triggered the psychological stress reaction. The glucocorticoids act in concert with the other stress signals (neuropeptides such as CRH, and neurotransmitters like norepinephrine) to tune the neural processes with physiological needs. The hormones affect selection of coping style, promote motivational processes and cognitive performance, with the goal to promote turning off the stress response while facilitating behavioral adaptation (see for reviews de Kloet et al. 2005; Lupien et al. 2009). Figure 11 shows these processes in detail.

Quote 1: Glucocorticoids are secreted under basal conditions in hourly pulses. The hormones are produced within minutes by the adrenal cortex, the pulse usually lasts about 20 min followed by a quiescent period until the next pulse arrives about an hour later. Studies using automatic frequent blood sampling showed that B pulses are increased in amplitude towards the activity period (i.e. the dark phase in rodents and light phase in humans), which contributes to the circadian rhythm. The blood pattern is reflected in the free hormone changes measured in the extracellular fluid in brain using microdialysis (Fig. 2). The pulses are thought to synchronize and coordinate daily activities and sleep-related events. Stress-induced glucocorticoid secretion is superimposed on the basal pulsatile and circadian rhythms.

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. Inflammatory disorders are characterized by increased frequency rather than amplitude of the ultradian rhythm. Accordingly, frequency encoding is an important modus operandi of the HPA axis. 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 (Lightman et al. 2020).

The pulse pattern appears crucial for the responsivity to stressors. We have demonstrated experimentally 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 (Sarabdjitsingh et al. 2010). See for mathematical modeling HPA axis (Spiga et al. 2017).

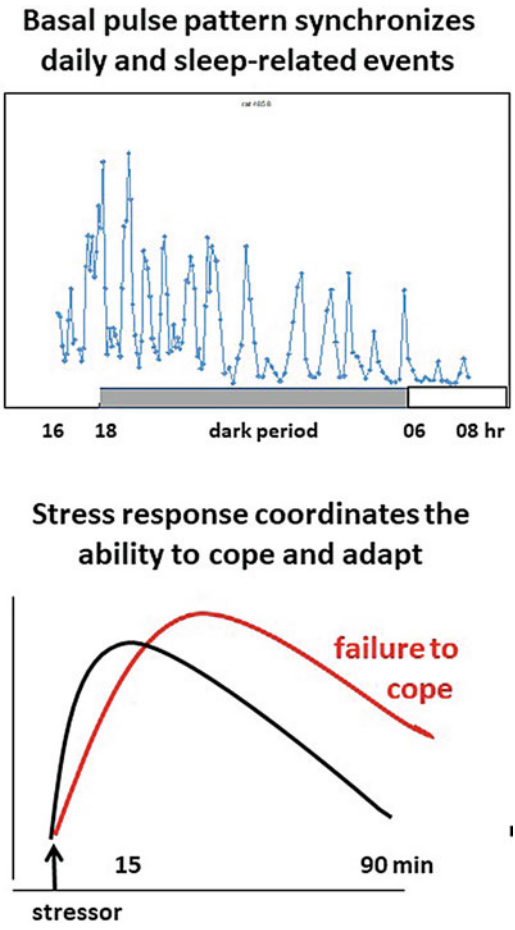

Fig. 2 Pulsatile (**a**) and stress-induced (**b**) corticosterone secretion. Note that a prolonged secretion of corticosterone occurs under conditions of failure to cope with stress

The question then is how responsiveness of glucocorticoid target genes in the 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 (nonpulsatile) 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. The experiments are described in Sarabdjitsingh et al. (2010).

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 McEwen's allostatic load condition. This is also a time of instability 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.

Quote 2: For medical science it is worth to examine how pulsatile glucocorticoid secretion 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 activate 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 when the endogenous hormone is insufficient to contain inflammatory or immune disorders. For instance, dexamethasone contains the "cytokine storm" evoked in some individuals by Covid-19.

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. 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 two receptor systems in the limbic brain: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), respectively (see Highlight 2).

> Quote 3: Glucocorticoids act as a double-edged sword in coordination of brain and behavior. The hormone permits an enhanced pulse and stress reaction via MR, which it subsequently suppresses via GR while promoting recovery, behavioral adaptation, and memory storage (see Highlight 2).

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), when administered to the ADX rat, was retained and accumulated in neurons of the hippocampus (McEwen et al. 1968). 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, when I worked as a postdoc in Bruce McEwen's laboratory in the early 1970s (de Kloet et al. 1975). 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 in an ATP-dependent fashion from the brain and prevents it from entering. 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 on F, which does not naturally occur in mouse, it appeared that F too is a substrate for Pgp explaining the reason why in humans 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 (Meijer et al. 1998; Dalm et al. 2019).

Determination of the concentration of both steroids in extracts of human post-mortem 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 in rodents, B and F in humans. 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 and F release was suppressed by Dex, the CRH synthesis and release was not suppressed, a finding that supported the concept that – counter intuitively – low doses of Dex can create a hypocorticoid state of the brain (Karssen et al. 2005).

> Quote 4: Dexamethasone poorly crosses (in rodents as well as humans) the blood–brain barrier because of multidrug resistance P glycoprotein, while the entrance of B into the brain is not hampered.

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

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. 3). Both receptor types occur as nuclear receptors that act as transcription factors in the regulation of gene expression implying that the onset is delayed to 30–60 min with a duration of several hours. Moreover, in particular GR has two modes of operation: trans-activation and transrepression, the latter involving interaction with other transcription factors that are induced by other signaling cascades. Interestingly, the receptors also mediate rapid non-genomic actions with an onset of seconds to minutes. Non-genomic MR activation promotes excitatory transmission. GR activation promotes endocannabinoid release which exerts trans-synaptically an inhibitory action on neurotransmitter release. These non-genomic actions are discussed in more detail under Highlight nr 4. See Fig. 4 action mechanism of the steroids.

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 35 years ago. I realized to have mined gold:

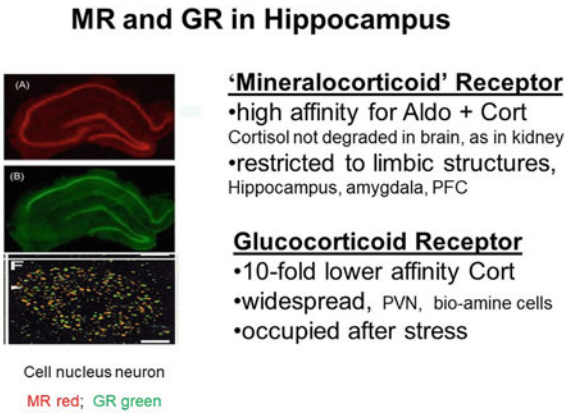

Fig. 3 MR and GR in the hippocampus. Co-localization of MR and GR in the hippocampus. The properties and localization of MR and GR are also described

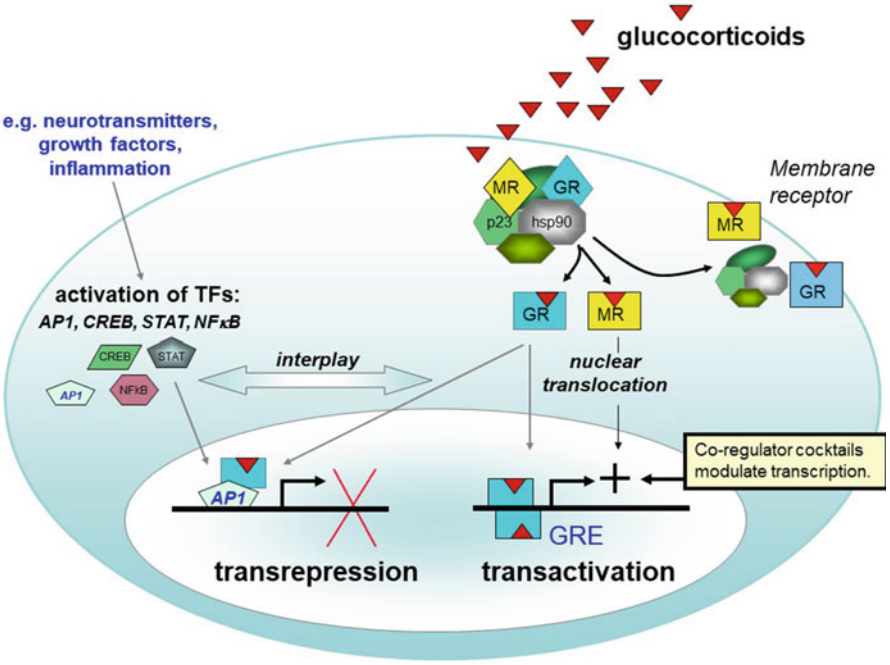

Fig. 4 Action mechanism of corticosteroids. Mechanism of glucocorticoid action. The receptors as part of a multimeric protein bind the glucocorticoids, which then dissociate and translocate to the cell nucleus. *MR* mineralocorticoid receptors, *GR* glucocorticoid receptors. MR and GR are transcription 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, NFkB). MR and GR can also function as membrane receptors modulating neurotransmission directly. (Courtesy of Dr N Datson)

one single stress hormone binding to two complementary receptor systems to account for basal and stressful regulations. The key paper by Reul and de Kloet, Endocrinology 1985 is still highly cited (Reul and de Kloet 1985).

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 (11-HSD2) that converts B into the inactive 11-dehydro-B congener. In brain, only the neurons of the n. tractus solitarii (NTS) express 11-HSD2 and are aldosterone selective. These neurons are important for the 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 11-HSD is expressed, which operates as a reductase and rather regenerates bioactive B from its inactive metabolite. Of note, the MR is promiscuous: the receptor binds not only aldosterone and B with high affinity, but also – albeit with lower affinity – progesterone and deoxycorticosterone. Because of the high affinity and the 100–1000-fold excess of B, MR "sees" predominantly B, including neurons in the NTS (Gasparini et al. 2019; de Kloet et al. 2018).

> Quote 5: The co-localization and properties of MR and GR in brain have been exploited for in-depth study of stress-coping and adaptation.

MR:GR Balance 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" (de Kloet et al. 1999, 2005, 2018, 2019).

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 are complementary in nature, timing, and direction with the goal to restore homeostasis.

MR:GR Balance in Neuroendocrine Regulation

Loss of function of MR by administering an antagonist systemically, intraventricularly, or locally in the hippocampus enhances basal pulsatility and stress-induced HPA axis activity. Upon chronic blockade of the MR this disinhibitory effect slowly disappears over a few days and eventually results in another set point of the HPA axis characterized by larger adrenals (ACTH stimulates also 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.

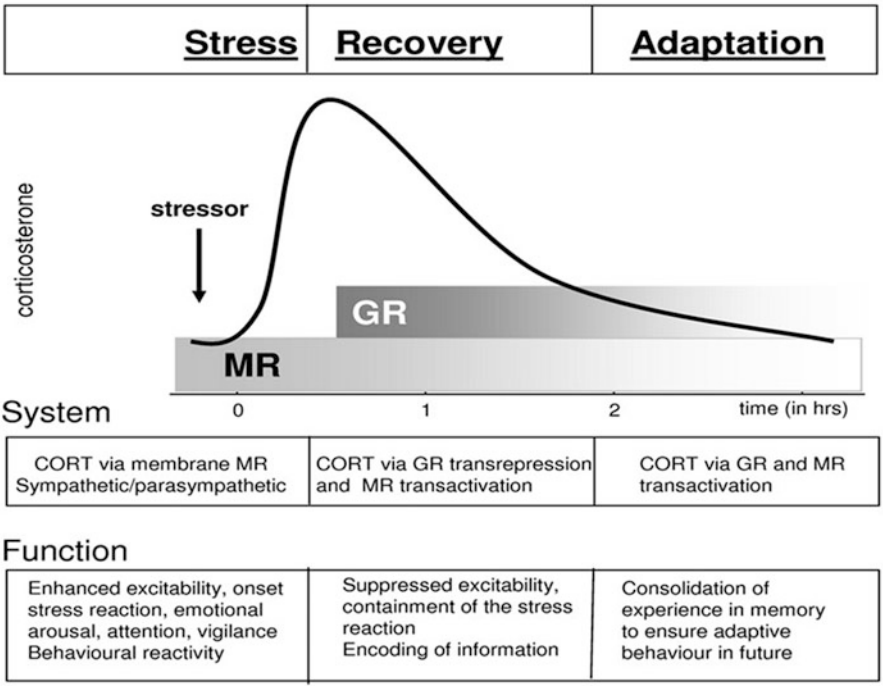

Fig. 5 The graph shows three phases, from the initial stress reaction to recovery and adaptation, during which molecular, cellular, and behavioral effects, exerted by CORT (B and F), occur. (Reprinted with permission from Oitzl et al. 2010)

Conversely, GR in the PVN and pituitary mediates as expected the direct negative feedback action on stress-induced HPA axis activity, which is disinhibited locally by GR blockade. The GR in hippocampus mediates effects opposite to those via MR on neuroendocrine regulation and autonomic outflow. See Fig. 5.

Relevance of MR:GR Balance for Behavior

The relevance of the MR:GR balance for behavior became apparent in a study by Melly Oitzl in the early 1990s using the well-known Morris water maze. 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. 6). 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 (Oitzl and de Kloet 1992).

Blocking the MR with a mineralocorticoid antagonist *after* the learning trial was ineffective in affecting the storage of information. To affect behavior the MR

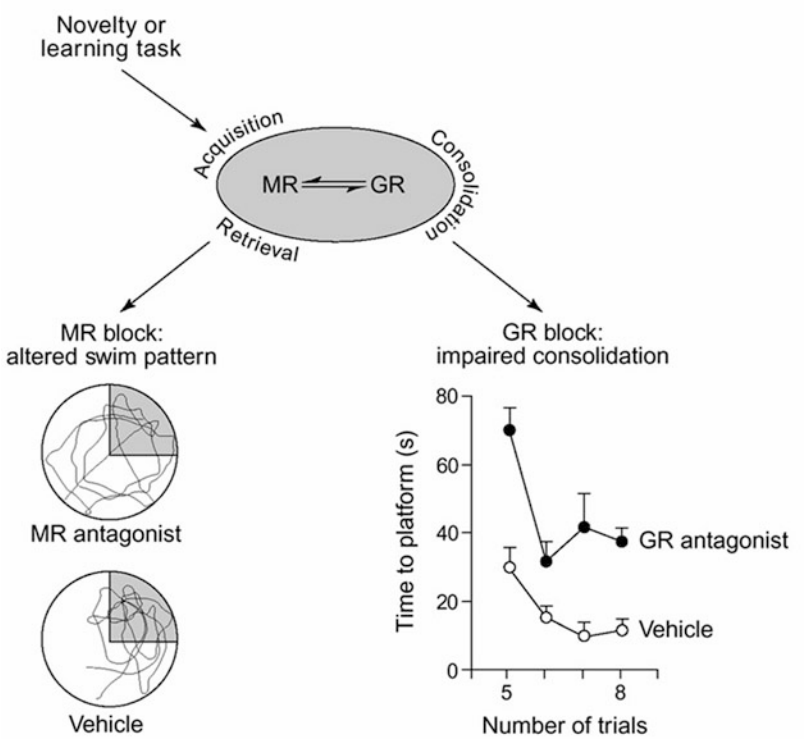

Fig. 6 MR and GR antagonists in the Morris Maze. Information processing modulated by CORT action via MR and GR in the Morris water maze. GR blockade with a glucocorticoid antagonist administered immediately after the learning session *blocks* consolidation of the learned swimming pattern measured 24 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)

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 capable of facilitating the switch between learning and memory systems in mice. In a series of studies by Schwabe and Oitzl et al. (2012) tests were designed to allow the mice to use either a caudate nucleus-based stimulus–response strategy (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 the spatial 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 to 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 highlights that a coordinated MR- and GR-mediated action is involved in memory storage and retrieval of stressful learning experience (Oitzl et al. 2012).

The enhanced perseveration of learned behavior suggesting either better recall or less flexibility upon stimulation of the MR observed in the above two maze tests was confirmed using genetically modified animals in a study by Harris et al. (2013). Mice that had forebrain overexpression of MR showed indeed perseverance in maze- and fear-learning paradigms particularly if together with GR underexpression. In addition, MR overexpression showed reduced HPA-axis activity after stress and at the circadian peak, and this effect was most pronounced in the animals that had additional GR underexpression.

As observed by Roozendaal, de Quervain, and McGaugh the glucocorticoid action in behavior facilitates a noradrenergic input in the limbic system (Roozendaal et al. 2009; de Quervain et al. 2017) The timing of glucocorticoid manipulation is an important determinant; both hormones should be present at roughly the same time. 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 corticosteroids (in the hippocampus) when applied in advance of noradrenaline. The latter is most likely not a physiological condition, since noradrenaline levels are increased prior to the peak of corticosterone. Interestingly, in the basolateral amygdala moderate concentrations of noradrenaline also suppress subsequent responses to corticosterone. However, when corticosterone is administered after noradrenaline at high concentrations – mimicking severely stressful conditions – amygdala cells remain excited for a long time (Karst and Joëls 2016). This shows that timing and concentration of the various stress mediators is important. 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 the onset of the stress response, selection of coping style, learning, and memory retrieval. Via GR the stress response is terminated, the selected coping style is contextualized and stored in memory for future use.

Gene Variants

Genetic variations have been identified in the MR and GR, as well as in proteins that determine their transcriptional activity (Fig. 7). Splice variants have been identified in the translated and untranslated regions of the receptors. Single nucleotide

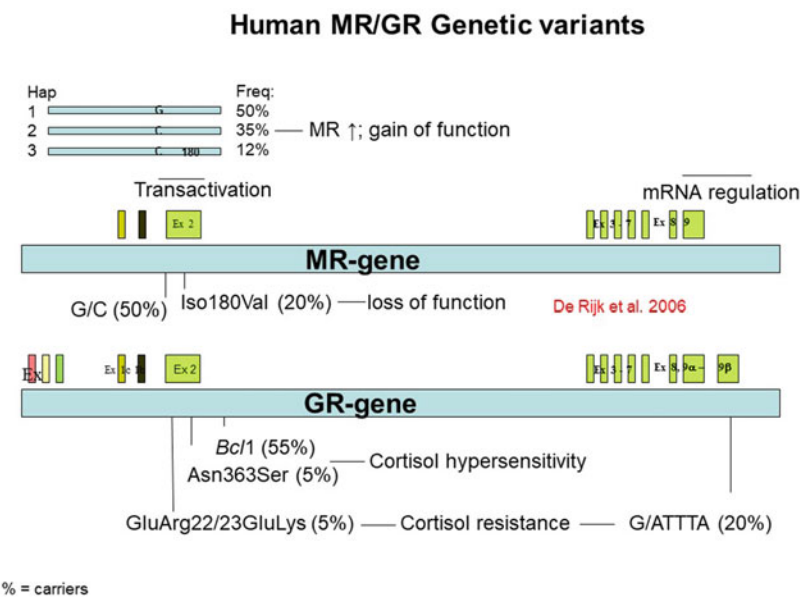

Fig. 7 Gene variants of MR and GR. An overview of the common genetic variants of the MR and GR and their effects on HPA axis reactivity. (Based on data from Derijk et al. 2008)

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.

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 Nienke 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/180 V) 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. 2011). 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 differences in MR expression in the brain, conferring interindividual variability in HPA axis reactivity and susceptibility to 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, etc. 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 (Fig. 8).

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), shows a highly comparable U-shaped dose dependency for B (see Fig. 8). 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 or that both pathways happen to be regulated in a similar fashion, independent of each other.

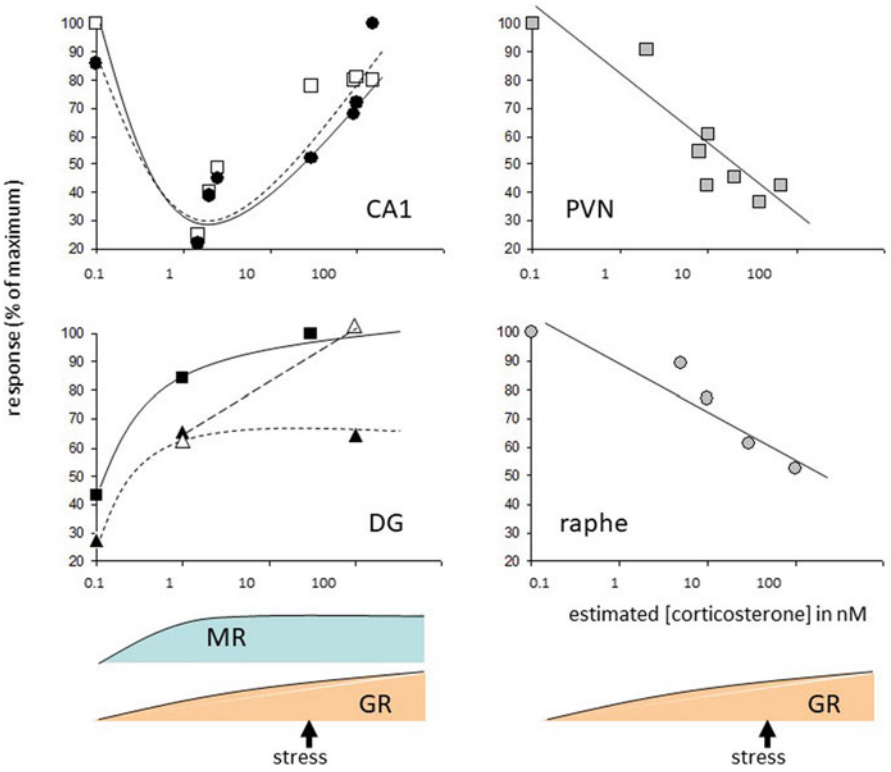

Fig. 8 U-shaped MR- and GR-mediated actions. Dose–response relationships for cellular effects of corticosterone in the CA1 hippocampal area, the dentate gyrus (*DG*), the paraventricular nucleus of the hypothalamus (*PVN*), and the dorsal raphe nucleus. The diagrams show hormone responses expressed as 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)

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 gyrus cells that 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 higher 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 stress-related 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 mEPSC 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, but rather saw a *decrease*. 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. 9). 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. See for overviews (Joëls et al. 2008, 2012, 2018; Joëls and Baram 2009; Joels et al. 2006).

> 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 true for other brain areas requires more investigation.

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

As early as in the 1957, the late 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

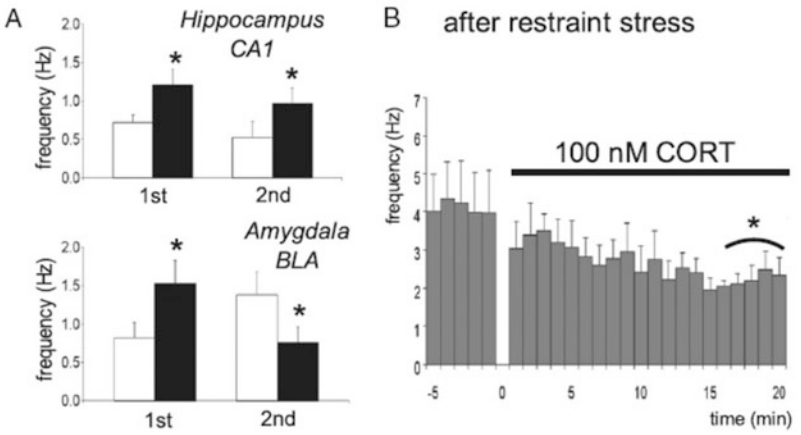

Fig. 9 Metaplasticity in the Basolateral Amygdala. In the presence of corticosterone (*black bars* in **a**) mEPSC frequency in the hippocampus is increased. A similar response is seen after a second pulse of corticosterone. In the BLA, however, the first pulse lastingly increases mEPSC frequency, which 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). See also (Karst et al. 2005)

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) the stress hyporesponsive period (SHRP): the effect of early experience, (2) later life outcome: three-hit hypothesis and the match-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. For instance, 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. This element, that is, the (predictability of the) dam's care of her litter, is also a key element of another frequently used model which involves the rearing of pups in an impoverished environment.

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

Adult rats that had received as pups very low amounts of maternal care showed enhanced emotional and stress reactivity coupled to 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 the late 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 strong phenotype characterized by the abolishment of PPI, a marker for sensorimotor gating, an impaired working memory,

and a defect in social interaction, which all are characteristic for patients suffering from schizophrenia (Daskalakis et al. 2013).

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*" Seymour Levine once noted when referring to the amazing plasticity of the brain (Levine 2005). 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. This depends on the genetic background, as has also been shown in humans (de Kloet and Oitzl 2003).

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 came from studies showing that malnutrition and stress experienced during pregnancy produce 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 et al. (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 embryos 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 experiments by Danielle Champagne and colleagues it was first demonstrated that adult 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. 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

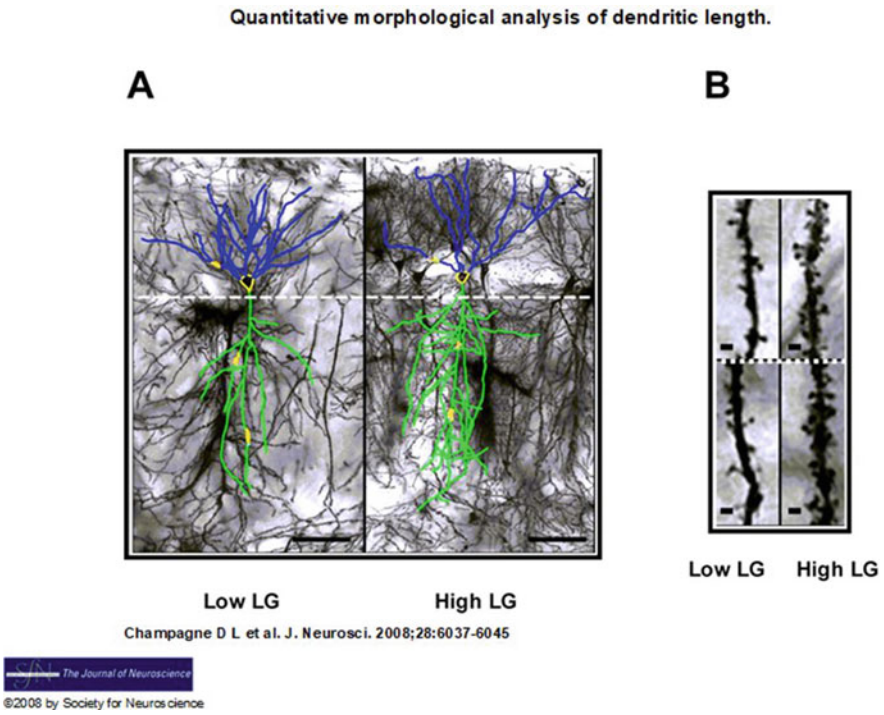

Fig. 10 Hippocampal CA1 morphology of low maternal care offspring. Effect of the quality and quantity of maternal care on dendrite structure of Golgi-stained hippocampal CA1 neurons of the 2-month-old offspring. LG, maternal licking and grooming. (From Champagne et al. 2008)

impaired (Fig. 10). However, if the low maternal care offspring was exposed to B (as a proxy for stressful conditions), 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 (Champagne et al. 2008).

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 "match-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 scenarios linked to either a 3-hit or a match-mismatch outcome which may lead in its extreme form to either an excellent or poor health.

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 the field of 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 now by others and with new techniques.

Neuroendocrinology Remains Alive and Kicking

Admittedly, great discoveries in the field of hormones took place 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 20 years ago, and neither Selye's "Stress" concept nor the Geoffrey Harris concept of neuroendocrine communication got awarded. Yet, Endocrinology has a big impact on the Biomedical Sciences. Why?

An interesting answer to this question can 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 70 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.

Context-Dependent Glucocorticoid Action

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 (Belsky et al. 2009).

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 (Datson et al. 2013).

Glucocorticoid action is complex. For instance, the GR is encoded by only one gene, but the protein occurs according to John Cidlowski in dozens of variants (Oakley and Cidlowski 2011). 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 (Picard and Sandi 2021).

Sex Differences

Most data on stress, coping, and adaptation refer to studies in males. Yet, the preferred coping strategy shows profound sex-differences. Males engage in the "fight-or-flight" response to gain control, while females rather rely on a more passive strategy classified as "tend-and-befriend" (Taylor et al. 2000), This is due the masculinizing "organizational" effects of testosterone during perinatal life which are in males further enhanced by androgens during puberty, when the female brain becomes responsive to sex steroids. Androgens stimulate and estrogens attenuate HPA-axis activity, and this difference is amplified during anxiety and depression in which males have higher cortisol secretion, while these disorders are female prevalent (Bale and Epperson 2017). This finding suggests that there is also a sex difference in glucocorticoid feedback on brain, which in humans indeed is sexual

dimorphic (Zorn et al. 2017). There are profound sex differences in the liver transcriptome in response to glucocorticoid stimulation. Males have excess glucocorticoid responsive genes not only in liver, but also in brain (Duma et al. 2010). Thus, the stress response system and glucocorticoid feedback are sexual dimorphic and this sex differences may originate from organizational actions of sex hormones. Interestingly, at a meta-analytical level adult behavioral effects of early life stress in rodents are much more pronounced in males than in females (Bonapersona et al. 2019). All of these observations warrant further in-depth research in male/female differences during stress-coping and adaptation through the entire lifespan.

Bottom-Up and Top-Down Control of Stress Coping

Figure 11 presents in a scheme how an adverse signal (the stressor) is processed in the brain and produces eventually a coping response that is stored in memory for future use as part of behavioral adaptation. First, the signal perceived novel and adverse triggers via ascending pathways arising from the brain stem an ALARM reaction guided by the *salience network* leading to arousal, vigilance, and alertness, while activating the sympathetic nervous system and the HPA-axis. At the same time APPRAISAL processes are activated involving *limbic network*s such as contextual

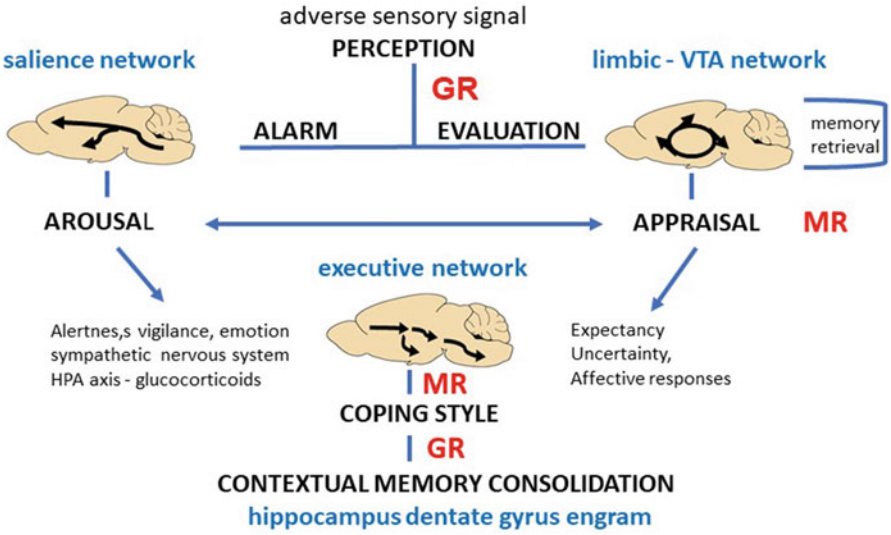

Fig. 11 Perceived novel information triggers alarm and appraisal processes ultimately leading to activation of the executive network underlying *top-down* control of stress-coping and behavioral adaptation. Glucocorticoids secreted by the adrenals as a result of HPA-axis activation driven by the salience network exert a *bottom-up* feedback action via MR and GR. Hippocampal MR is involved in contextual retrieval of memory for selection of habitual vs declarative coping styles. GR activation promotes emotions, motivational processes, and contextual memory consolidation and there is some evidence for a role in perception of sensory information (Henkin and Daly, 1968; de Kloet et al, 2019; Herman et al. 2020; Lingg et al. 2020; Obleser et al. 2021; Molendijk and de Kloet, 2021).

memory retrieval in *hippocampus*, valuation in the *mesocortical dopaminergic circuitry* and emotions in *amygdala*. Outcome can be, for example, expectancy or uncertainty. Alarm and appraisal processes interact in modulating the *executive network* originating from neuronal ensembles in the mPFC for selection of COPING STYLE to enable control. This executive network is excitatory in activating hubs that direct downstream GABA-ergic inhibitory control over the HPA axis and the peri-aqueductal gray resulting in active or passive coping. If coping is successful, the parasympathetic nervous system is activated and the HPA axis is suppressed in activity, and adaptation promoted (Radley and Johnson 2018; Douma and de Kloet 2020). If coping fails, the whole cascade from alarm to appraisal to executive mPFC control is reinforced.

The mPFC, thus, exerts *top-down* control over executive network that governs stress-coping and adaptation. The glucocorticoids, as alarm (stressor)-induced end product of the HPA-axis, synergize with appraisal and executive network activity. The circuits are informed by B and F in *bottom-up* fashion about physiological needs and energy allocation. This chapter focused on research in the past decades, which has generated quite some progress in understanding how these circulating glucocorticoid hormones act in hippocampal circuits that harbor cognitive performance related to space and context: glucocorticoids promote contextual memory consolidation and retrieval. The new data have provided more insight into the mechanism underlying the B and F-enhanced excitability that is mediated by membrane and nuclear MR on the one hand and the GR-mediated suppression of transiently raised excitability on the other and have a role in dentate gyrus neurogenesis. On the cellular, circuit, and behavioral level data were generated supporting the MR:GR balance hypothesis such that MR activation promotes selection of coping style and GR memory consolidation. 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 affective features related to coping with stressful information (de Kloet et al. 2019; Molendijk and de Kloet 2021).

Mental Health and Quality of Life

Whatever novel technical approach is chosen to study stress in the brain, 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, health means an efficient transition of allostatic states over the fitness landscape to readily restore homeostasis upon disturbance. 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 (Holsboer and Ising 2010). Briefly: a strategy aimed to advance the twenty-first-century Neurobiology of Mental Health.

References

Bale TL, Epperson CN (2017) Sex as a biological variable: who, what, when, why, and how. Neuropsychopharmacology 42:386–396. https://doi.org/10.1038/npp.2016.215. Epub 2016 Sep 23. PMID: 27658485; PMCID: PMC5399243

Belsky J, Jonassaint C, Pluess M, Stanton M, Brummett B, Williams R (2009) Vulnerability genes or plasticity genes? Mol Psychiatry 14:746–754

Bonapersona V, Kentrop J, Van Lissa CJ, van der Veen R, Joëls M, Sarabdjitsingh RA (2019) The behavioral phenotype of early life adversity: a 3-level meta-analysis of rodent studies. Neurosci Biobehav Rev 102:299–307. https://doi.org/10.1016/j.neubiorev.2019.04.021. Epub 2019 Apr 29. PMID: 31047892

Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, Joëls M, Krugers H (2008) Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. J Neurosci 28:6037–6045

Dalm S, Karssen AM, Meijer OC, Belanoff JK, de Kloet ER (2019) Resetting the stress system with a mifepristone challenge. Cell Mol Neurobiol 39(4):503–522. https://doi.org/10.1007/s10571-018-0614-5. Epub 2018 Sep 1. PMID: 30173378; PMCID: PMC6469632

Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER (2013) The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. Psychoneuroendocrinology 38:1858–1873. https://doi.org/10.1016/j.psyneuen.2013.06.008. Epub 2013 Jul 7. PMID: 23838101; PMCID: PMC3773020

Datson NA, van den Oever JM, Korobko OB, Magarinos AM, de Kloet ER, McEwen BS (2013) Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. Endocrinology 154(9): 3261–3272. https://doi.org/10.1210/en.2012-2233. Epub 2013 Apr 30. PMID: 23633533; PMCID: PMC3749472

de Kloet ER, Oitzl MS (2003) Who cares for a stressed brain? The mother, the kid or both? Neurobiol Aging 24(Suppl 1):S61–S65; discussion S67–8. PMID: 12829110. https://doi.org/10.1016/s0197-4580(03)00057-5

De Kloet R, Wallach G, McEwen BS (1975) Differences in corticosterone and dexamethasone binding to rat brain and pituitary. Endocrinology 96:598–609. https://doi.org/10.1210/endo-96-3-598. PMID: 163728

de Kloet ER, Oitzl MS, Joëls M (1999) Stress and cognition: are corticosteroids good or bad guys? Trends Neurosci 22:422–426

de Kloet ER, Joëls M, Holsboer F (2005) Stress and the brain: from adaptation to disease. Nat Rev Neurosci 6:463–475

de Kloet ER, Meijer OC, de Nicola AF, de Rijk RH, Joëls M (2018) Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuroinflammation. Front Neuroendocrinol 49:124–145. https://doi.org/10.1016/j.yfrne.2018.02.003. Epub 2018 Feb 8. PMID: 29428549

de Kloet ER, de Kloet SF, de Kloet CS, de Kloet AD (2019) Top-down and bottom-up control of stress-coping. J Neuroendocrinol 31:e12675. https://doi.org/10.1111/jne.12675. Epub 2019 Feb 1. PMID: 30578574; PMCID: PMC6519262

de Quervain D, Schwabe L, Roozendaal B (2017) Stress, glucocorticoids and memory: implications for treating fear-related disorders. Nat Rev Neurosci 18(1):7–19. https://doi.org/10.1038/nrn.2016.155. Epub 2016 Nov 24. PMID: 27881856

Derijk RH, van Leeuwen N, Klok MD, Zitman FG (2008) Corticosteroid receptor-gene variants: modulators of the stress-response and implications for mental health. Eur J Pharmacol 585: 492–501

Douma EH, de Kloet ER (2020) Stress-induced plasticity and functioning of ventral tegmental dopamine neurons. Neurosci Biobehav Rev 108:48–77. https://doi.org/10.1016/j.neubiorev.2019.10.015. Epub 2019 Oct 27. PMID: 31666179

Duma D, Collins JB, Chou JW, Cidlowski JA (2010) Sexually dimorphic actions of glucocorticoids provide a link to inflammatory diseases with gender differences in prevalence. Sci Signal 3(143):ra74. https://doi.org/10.1126/scisignal.2001077. Erratum in: Sci Signal. 2010;3(145): er10. PMID: 20940427; PMCID: PMC4772900

Gasparini S, Resch JM, Narayan SV, Peltekian L, Iverson GN, Karthik S, Geerling JC (2019) Aldosterone-sensitive HSD2 neurons in mice. Brain Struct Funct 224:387–417. https://doi.org/10.1007/s00429-018-1778-y. Epub 2018 Oct 20. PMID: 30343334; PMCID: PMC6369013

Harris AP, Holmes MC, de Kloet ER, Chapman KE, Seckl JR (2013) Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour. Psychoneuroendocrinology 38(5): 648–658. https://doi.org/10.1016/j.psyneuen.2012.08.007. Epub 2012 Sep 11. PMID: 22980941

Herman JP, Nawreen N, Smail MA, Cotella EM (2020) Brain mechanisms of HPA axis regulation: neurocircuitry and feedback in context Richard Kvetnansky lecture. Stress 23(6):617–632. https://doi.org/10.1080/10253890.2020.1859475. Epub 2020 Dec 21. PMID: 33345670; PMCID: PMC8034599.

Hermans EJ, Henckens MJ, Joëls M, Fernández G (2014) Dynamic adaptation of large-scale brain networks in response to acute stressors. Trends Neurosci 37(6):304–14. https://doi.org/10.1016/j.tins.2014.03.006. Epub 2014 Apr 21. PMID: 24766931.

Henkin RI, Daly RL (1968) Auditory detection and perception in normal man and in patients with adrenal cortical insufficiency: effect of adrenal cortical steroids. J Clin Invest 47:1269–1280. https://doi.org/10.1172/JCI105819. PMID: 4298249; PMCID: PMC297283

Holsboer F, Ising M (2010) Stress hormone regulation: biological role and translation into therapy. Annu Rev Psychol 61(81–109):C1–C11

Ingle DJ (1938) Discovery of feedback. Am J Phys 124:369–371

Joëls M (2006) Corticosteroid effects in the brain: U-shape it. Trends Pharmacol Sci 27:244–250

Joëls M, Baram TZ (2009) The neuro-symphony of stress. Nat Rev Neurosci 10:459–466

Joëls M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ (2006) Learning under stress: how does it work? Trends Cogn Sci 10:152–158

Joëls M, Karst H, deRijk R, de Kloet ER (2008) The coming out of the brain mineralocorticoid receptor. Trends Neurosci 31:1–7

Joëls M, Sarabdjitsingh RA, Karst H (2012) Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. Pharmacol Rev 64:901–938. https://doi.org/10.1124/pr.112.005892

Joëls M, Karst H, Sarabdjitsingh RA (2018) The stressed brain of humans and rodents. Acta Physiol (Oxf) 223:e13066. https://doi.org/10.1111/apha.13066. Epub 2018 Apr 16. PMID: 29575542; PMCID: PMC5969253

Karssen AM, Meijer OC, Berry A, Sanjuan Piñol R, de Kloet ER (2005) Low doses of dexamethasone can produce a hypocorticosteroid state in the brain. Endocrinology 146:5587–5595. https://doi.org/10.1210/en.2005-0501. Epub 2005 Sep 8. PMID: 16150912

Karst H, Joëls M (2016) Severe stress hormone conditions cause an extended window of excitability in the mouse basolateral amygdala. Neuropharmacology 110:175–180. https://doi.org/10.1016/j.neuropharm.2016.07.027. Epub 2016 Jul 25. PMID: 27460963

Karst H, Berger S, Turiault M, Tronche F, Schütz G, Joëls M (2005) Mineralocorticoid receptors are indispensible for nongenomic modulation of hippocampal glutamate transmission by cortiocsterone. Proc Natl Acad Sci USA 102:19204–19207

Karst H, Berger S, Erdmann G, Schütz G, Joëls M (2010) Metaplasticity of amygdalar responses to the stress hormone corticosterone. Proc Natl Acad Sci USA 107:14449–14454

Klok MD, Giltay EJ, Van der Does AJW, Geleijnse JM, Antypa N, Penninx BWJH, de Geus EJC, Willemsen G, Boomsma DI, Van Leeuwen N, Zitman FG, de Kloet ER, de Rijk RH (2011) A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. Transl Psychiatry 1:e62

Krugers HJ, Hoogenraad CC, Groc L (2010) Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. Nat Rev Neurosci 11:675–681

Levine S (2005) Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology 30:939–994

Lightman SL, Birnie MT, Conway-Campbell BL (2020) Dynamics of ACTH and cortisol secretion and implications for disease. Endocr Rev 41:470–490. https://doi.org/10.1210/endrev/bnaa002. PMID: 32060528; PMCID: PMC7240781

Lingg RT, Johnson SB, Emmons EB, Anderson RM, Romig-Martin SA, Narayanan NS, McGaugh JL, LaLumiere RT, Radley JJ (2020) Bed nuclei of the stria terminalis modulate memory consolidation via glucocorticoid-dependent and -independent circuits. Proc Natl Acad Sci U S A 117(14):8104–8114. https://doi.org/10.1073/pnas.1915501117. Epub 2020 Mar 19. PMID: 32193346; PMCID: PMC7148582.

Lupien SJ, McEwen BS, Gunnar MR, Heim C (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 10:434–445

McEwen BS, Wingfield JC (2010) What is in a name? Integrating homeostasis, allostasis and stress. Horm Behav 57:105–111

McEwen BS, Weiss JM, Schwartz LS (1968) Selective retention of corticosterone by limbic structures in rat brain. Nature 220:911–912. https://doi.org/10.1038/220911a0. PMID: 4301849

Meijer OC, de Lange EC, Breimer DD, de Boer AG, Workel JO, de Kloet ER (1998) Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology 139:1789–1793. https://doi.org/10.1210/endo.139.4.5917. PMID: 9528963

Molendijk ML, de Kloet ER (2021) Forced swim stressor: trends in usage and mechanistic consideration. Eur J Neurosci. https://doi.org/10.1111/ejn.15139. Epub ahead of print. PMID: 33548153

Munck A, Guyre PM, Holbrook NJ (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5, 25–44. https://doi.org/10.1210/edrv-5-1-25.

Oakley RH, Cidlowski JA (2011) Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. J Biol Chem 286:3177–3184

Obleser J, Kreitewolf J, Vielhauer R, Lindner F, David C, Oster H, Tune S (2021) Circadian fluctuations in glucocorticoid level predict perceptual discrimination sensitivity. iScience. 24 (4):102345. https://doi.org/10.1016/j.isci.2021.102345. PMID: 33870139; PMCID: PMC8047178.

Oitzl MS, de Kloet ER (1992) Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. Behav Neurosci 106:62–71

Oitzl MS, Champagne DL, van der Veen R, de Kloet ER (2010) Brain development under stress: hypotheses of glucocorticoid actions revisited. Neurosci Biobehav Rev 34:853–866

Oitzl MS, Schwabe L, Aggleton JP (2012) Memory formation: its changing face. Neurosci Biobehav Rev. [Epub ahead of print]

Picard M, Sandi C (2021) The social nature of mitochondria: implications for human health. Neurosci Biobehav Rev 120:595–610. https://doi.org/10.1016/j.neubiorev.2020.04.017. Epub 2020 Jul 8. PMID: 32651001

Radley JJ, Johnson SB (2018) Anteroventral bed nuclei of the stria terminalis neurocircuitry: towards an integration of HPA axis modulation with coping behaviors – Curt Richter Award Paper 2017. Psychoneuroendocrinology 89:239–249. https://doi.org/10.1016/j.psyneuen.2017.12.005. Epub 2017 Dec 24. PMID: 29395488; PMCID: PMC5878723

Reul JM, de Kloet ER (1985) Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 117:2505–2511. https://doi.org/10.1210/endo-117-6-2505. PMID: 2998738

Roozendaal B, McEwen BS, Chattarji S (2009) Stress, memory and the amygdala. Nat Rev Neurosci 10:423–433

Sarabdjitsingh RA, Isenia S, Polman A, Mijalkovic J, Lachize S, Datson N, de Kloet ER, Meijer OC (2010) Disrupted corticosterone pulsatile patterns attenuate responsiveness to glucocorticoid signaling in rat brain. Endocrinology 151:1177–1186

Sibug RM, de Koning J, Tijssen AM, de Ruiter MC, de Kloet ER, Helmerhorst FM (2005) Urinary gonadotrophins but not recombinant gonadotrophins reduce expression of VEGF120 and its

receptors flt-1 and flk-1 in the mouse uterus during the peri-implantation period. Hum Reprod 20:649–656

Spiga F, Zavala E, Walker JJ, Zhao Z, Terry JR, Lightman SL (2017) Dynamic responses of the adrenal steroidogenic regulatory network. Proc Natl Acad Sci USA 114:E6466–E6474. https://doi.org/10.1073/pnas.1703779114. Epub 2017 Jul 17. PMID: 28716938; PMCID: PMC5547620

Tausk M, 1952. Hat die Nebennierrinde tatsächlich eine Verteidigungsfunktion? Das Horm. 3

Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA (2000) Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. Psychol Rev 107(3): 411–429. https://doi.org/10.1037/0033-295x.107.3.411. PMID: 10941275

Zorn JV, Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH (2017) Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. Psychoneuroendocrinology 77:25–36. https://doi.org/10.1016/j.psyneuen.2016.11.036. Epub 2016 Dec 8. PMID: 28012291