HORMONES, BRAIN AND STRESS

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Abstract. The stress system orchestrates body and brain responses to the environment. This action exerted by the mediators of the stress system has two modes of operation. The immediate response mode driven by corticotropin-releasing hormone (CRH) organises via CRH-1 receptors the behavioural, sympathetic and hypothalamic-pituitary-adrenal (HPA) responses to a stressor. In the other - slower - mode, which facilitates behavioural adaptation, the urocortins acting through CRH-2 receptors seem prominent. Corticosteroid hormones secreted by the adrenal cortex are implicated in both modes through their high affinity type 1 (mineralocorticoid receptors - MR) and lower affinity type 2 (glucocorticoid receptors - GR) receptors that are co-localised in limbic neural circuitry. Current data suggest that MR controls in specific afferents the threshold or sensitivity of the fast CRH-1 driven stress system mode and thus prevents disturbance of homeostasis, while GR facilitates its recovery by restraining in these very same circuits stress responses and by mobilising energy resources. In preparation for future events GR facilitates behavioural adaptation and promotes storage of energy. The balance in the two stress system modes is thought to be essential for cell homeostasis, mental performance and health. Imbalance induced by genetic modification or chronic stressors changes specific neural signalling pathways underlying psychic domains of cognition and emotion, anxiety and aggression. This Yin-Yang stress concept is fundamental for genomic strategies to understand the mechanistic underpinning of cortisol-induced stress-related disorders such as i.e. severe forms of depression and co-morbid diseases.

Keywords: Stress – Behaviour – Corticosteroids – Brain - Genes

Introduction

It is a great honour to be invited as introductory lecturer for the triennial conference on catecholamines and other transmitters in stress. While preparing for this 8th edition of the Smolenice Castle Conference I sat back for a moment reflecting on the very first Conference more than twenty years ago. I see the castle's facilities, the atmosphere and the hospitality, images that stand out so characteristic in the Slovakian hills. Images that trigger the memory of research life at that time. No laptop, fax and e-mail. Corrections and literature references were endless, tedious jobs. Ima-ges that also give me a chance to start from a historical perspective, to phrase fundamental questions and to sketch some future directions, at least towards June 2006. Images reminding me that at each Conference the most imminent question asked by the participants is: when will the next meeting be Richard?

A fundamental question for the castle meeting is how the stress system can change its function from protection to damage and disease. It is common knowledge that the duration and nature of the stressor are important determinants, but then the question remains why one individual copes

and remains in excellent health, while the other suffers and breaks down under similar adverse conditions. Current wisdom predicts an effective stress reaction as a healthy condition. "Effective" meaning a highly reactive system that readily turns on and off its behavioural, autonomous and endocrine responses to stressors. If the system responds sluggish, or when stress reactions are slowly terminated and prolonged, its mediators enhance vulnerability to damage and disease, for which the individual is predisposed. On the genomic level this implies that it is essential to understand how transient changes in expression of stress responsive genes are converted to prolonged maladaptive genomic changes (SABBAN and KVETNANSKY 2001).

In the control of stress reactions the corticosteroid hormones operating in concert with catecholamines and other transmitter are very potent players. If corticosteroid control is insufficient stress reactions are much too strong (HEIM et al. 2000). Alternatively, if adaptation to stress fails circulating corticosteroid levels remain elevated for a prolonged period of time (SELYE 1952). This excess corticosteroid has catabolic consequences and leads to breakdown of vital functions (SAPOLSKY et al. 2000). At least 50% of the depressed patients have elevated cortisol due to feedback resistance and a flattened circadian rhythm in the face of sympathetic hyperactivity (BELANOFF et al. 2001; HOLSBOER 2000; 2001; CHROUSOS and GOLD 1992; GOLD and CHROUSOS 1999). These include the patients suffering from melancholic depression as opposed to the pathophysiological mirror image, the atypical depressed patient with the signs and symptoms of hypocortisolemia (GOLD and CHROUSOS 1999). Hypercorticism is in particular often a hallmark of severe depression with psychotic features (SCHATZBERG et al. 1985). The psychotically depressed patient appears to respond favourably to anti-glucocorticoid therapy (BELANOFF et al. 2002) as has been previously reported for Cushing patients (VAN DER LELY et al. 1991). If further validated (GOLD et al. 2002), the finding actually represents the first pathophysiological substrate for a psychiatric disorder caused by excess cortisol that can be rescued by anti-glucocorticoids.

Thus, the initial question on the role of the stress system can be rephrased to cortisol: how does its action change from protective into harmful. What is the cause and what are the consequences? To address these questions, I first discuss the action mechanism of the corticosteroids. These hormones act conditional, and accordingly the context they operate is extremely important. Second, some animal models for depression are discussed in which the stress system is dysregulated and I focus briefly on two representative models: mice genetically selected for extreme differences in coping style and mice subjected to early trauma. Third, new strategy's to identify novel molecular targets in stress circuitry for treatment of stress-related disorders are embedded in the section future directions. Most studies are in the mouse and rat that have corticosterone as the principal naturally occurring glucocorticoid, while man has cortisol.

Brain corticosteroid receptors as stress system nodes that integrate information in binary fashion

Steroid molecules can affect cell function through direct interactions with transmitter receptors, as is the case for instance with neurosteroids derived from progesterone and deoxycorticosterone that interact with the GABA receptor. Here we focus on the intracellular receptors that mediate a variety of signalisations. Activation can have immediate responses through re-aggregation and molecular remodelling of the receptor molecule in the cytoplasmic compartment or through direct interaction with the transcription machinery (BAULIEU 2000; AUPHAN et al. 1995).

Discovery of brain corticosteroid receptors. I started my studies in Holland when Bruce McEwen (MCEWEN et al. 1968) discovered corticosteroid receptors in the limbic brain. Much to the surprise of the established stress community these nuclear receptors were expressed in abundance in the hippocampus beyond the core of the hypothalamic-pituitary-adrenal (HPA) axis in the hypothalamic paraventricular nucleus (PVN) and the pituitary corticotrophs in which these receptors were expected. As a student my very first ex-

periment was with dexamethasone because in my opinion the much higher potency of the synthetic glucocorticoid would label the hippocampus even better than corticosterone. It did not, but rather labelled the pituitary corticotrophs and to some extent the CRH neurons in the PVN (DE KLOET et al.1975). Only years later we found out why. I give three intriguing twists in the story.

Multidrug resistance P-glycoprotein (mdr Pgp) is a gatekeeper for brain penetration. Dexamethasone in tracer amounts poorly labelled the brain (MEIJER et al. 1998). This is because mdr Pgp and related proteins in the blood-brain-barrier export circulating synthetic steroid after penetration in the endothelial cells out of the brain as it does with many exogenous compounds. While this was suspected many years ago the proof came from experiments with Pgp knockout mice. ^{3}H-Dexamethasone administered to mdr Pgp mutants accumulates in brain in a tenfold larger amount than in the wild types, and neurons of the hippocampus now weakly retain this steroid. Also ^{3}H-cortisol, not naturally occurring in rat and mouse, is poorly retained in wild type brains and appears a Pgp substrate. In the mutants profound labelling of hippocampal neurons occurs with cortisol, as is the case with corticosterone. To our surprise human MDR also recognises cortisol rather than corticosterone as substrate, and liquid chromatograph — mass spectrometry analysis of post-mortem human brain samples revealed that in fact corticosterone is the preferred brain corticosteroid (KARSSEN et al. 2001). One of the implications of the steroid gatekeeper is that moderate amounts of dexamethasone suppress stress-induced HPA activity at a pituitary rather than a brain site. Because of this pituitary HPA blockade body and brain are depleted of endogenous corticosterone. In the periphery glucocorticoid actions of corticosterone are substituted by dexamethasone, but the corticosterone-depleted brain is not. Hence, moderate amounts of dexamethasone produce an adrenalectomy-like state in the brain.

Mineralocorticoid and glucocorticoid receptors: co-localised binary system. Even in the mdr mutants dexamethasone does not label the limbic neurons as well as corticosterone does. We discovered that while both steroids are glucocorticoids, dexamethasone binds as expected with high affinity to the glucocorticoid receptor (GR); corticosterone prefers with highest affinity the mineralocorticoid receptors (MR) and has a ten fold lower affinity to GR (REUL and DE KLOET 1985). Hence in our original experiments corticosterone labelled the MR, but the tracer was too low in quantity to see the classical dexamethasone labelled GR. This discovery was possible because of the synthesis of "pure" glucocorticoids and the cloning of GR and MR (EVANS and ARRIZA 1989). The precise topography was revealed in brain with immunocytochemistry and in situ hybridisation (FUXE et al. 1985; VAN EEKELEN et al. 1988, ITO et al. 2000). With confocal microscopy MR and GR appeared to be co-localised in abundance in limbic neurons including hippocampal CA1 and dentate gyrus (VAN STEENSEL et al. 1995). Co-localisation occurred also in nuclei of the amygdala and medial prefrontal cortex (HELM et al. 2002), areas that have an important function in emotion and cognition.

MR binds aldosterone and corticosterone with similar affinity, yet in epithelial cells MR is aldosterone selective. In epithelial cells such as in kidney and the periventricular brain areas the enzyme 11-βHydroxysteroid dehydrogenase type 2 (HSD-2) oxidises corticosterone to the inactive 11-dehydro metabolite leaving these MR available only for aldosterone (EDWARDS et al. 1988). Hence these sites are aldosterone-selective in control of electrolyte homeostasis. Limbic brain regions do not express HSD-2 and therefore MR *sees* predominantly corticosterone that circulates in a 100 - 1000 fold excess. Rather a brain structure as the hippocampus contains HSD-1 which re-activates bioinactive corticosterone. Potentially this regeneration of corticosterone could lead under some circumstances to excess corticosteroid exposure and disease (SECKL 1997). This has been demonstrated with a mouse mutant with over-expressed HSD-1 in adipose tissue. The mutants developed visceral obesity with a high fat diet as well as insulin resistant diabetes, hyperlipedemia and hyperphagia. Thus 11-HSD may be common modulator of obesity and metabolic syndrome (MASUZAKI et al. 2001).

Receptors, transcription factors and co-regulators: the integrating pathway node. Some 8 years ago it was demonstrated that corticosteroid receptors could affect signalling pathways beyond activation of glucocorticoid response elements (GREs) by interaction with other transcription factors (AUPHAN et al. 1995). MR and GR both bind to these GREs, but only GR is capable to interact with transcription factors such as activating protein (AP-1) and nuclear factor κB (NFκB) to attenuate stress-induced signalisations through the membrane. This finding provided a firm mechanistic underpinning to the concept advanced by TAUSK (1951) and MUNCK et al. (1984) that glucocorticoids actually restrain primary stress reactions. It is now known that they achieve this restraint through interaction of the GR monomers with transcription factors driven by catecholamines and other transmitters.

Recently, co-activator and co-repressor molecules were identified that appeared powerful modulators of nuclear receptor function (MEIJER, 2002). Members of the steroid co-activator receptor (SRC) family of proteins promote agonist-induced receptor activation by permitting recruitment of e.g. CBP/p300 transcription activators and chromatin remodelling. The NCOR and SMRT co-repressor molecules do the opposite and promote repression of gene transcription. Current studies have shown uneven co-localisation of various SRC subtypes with MR and GR in brain suggesting some degree of cell specificity. *In vitro* transfection experiments suggest that variable stoechiometry of co-repressors and co-activators, either induced or congenital, underlies differential MR/GR functioning. The GR antagonist mifepristone (RU 486) with the receptors provides an interesting example of the possible modes of interaction with steroid receptor signalling. The antagonist may even act as an agonist bound to GR monomers in interaction with NFκB. It only acts as antagonist at GREs if sufficient co-repressor is available (figure 1).

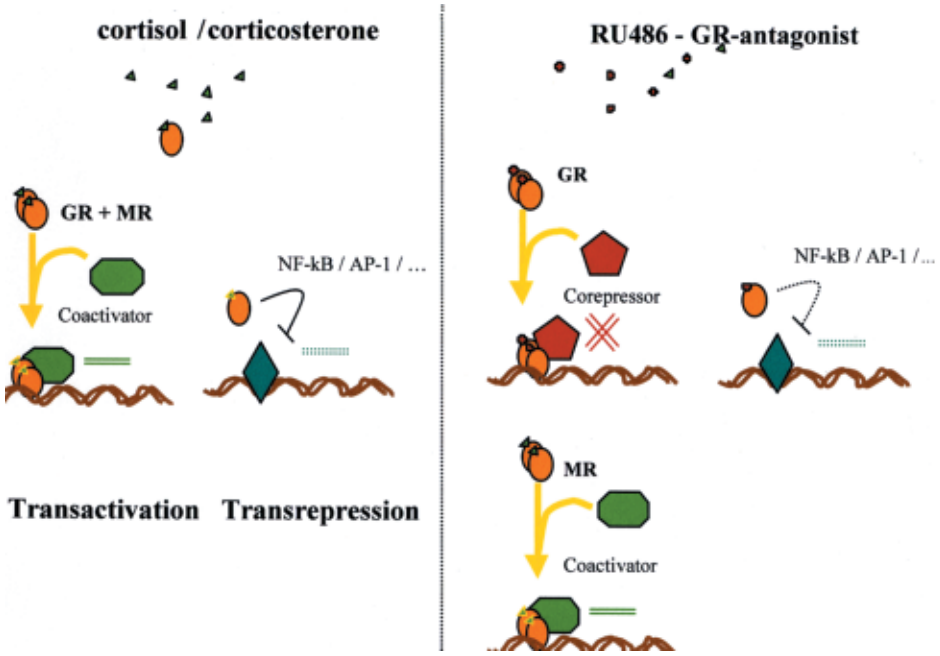

Fig 1 Action mechanism of corticosteroid hormones.
LEFT: **The naturally occurring glucocorticoid agonists cortisol and corticosterone interact with mineralocorticoid and glucocorticoid receptors (MR and GR), which upon dimerisation recruits co-activators to stimulate gene transcription (transactivation). As monomers GR, but not MR, can interfere with activation of gene transcription by transcription factors (NFκB / AP-1) triggered by membrane signalisations (transrepression).**
RIGHT: **GR dimerises upon glucocorticoid antagonist RU 486 and recruits co-repressors for blockade of agonist stimulated gene transcription. MR is not affected and escapes blockade, as does transrepression involving GR monomers and transcription factors. Courtesy of dr Onno Meijer.**

MR and GR operate in co-ordinate bi-directional fashion at the on- and offset of the stress response

Once properties, localisation and regulation were known we began detailed studies to explore the function on various levels of complexity. The approaches ranged from molecular changes to cellular homeostasis and adaptation to stress with the perspective to learn more about their implication on health and disease. We identified that one hormone (corticosterone) used two signalling systems (MR and GR). This work was inspired by Hans Selye's thesis on pro- and anti-inflammatory activity of mineralocorticoid and glucocorticoid hormones that were considered the exponents of opposing hormone actions crucial for maintenance of homeostasis. The work and viewpoints of TAUSK (1951), INGLE (1954), MUNCK et al. (1984) further highlighted the role of glucocorticoids in stress. It was common knowledge that any condition that (threatens) to disturb homeostasis triggered primary stress reactions that were prevented from overshooting by GR-mediated effects requiring stress levels of corticosterone. Thus inflammation, infection, energy need and psychological challenges all trigger via stressor-specific pathways the "non-specific" HPA response, and glucocorticoids feedback to facilitate elimination of the primary stress reaction. If the inflammation subsides the source of the stressor is eliminated.

An elegant demonstration of this principle was provided by Dallman and colleagues (LAUGERO et al. 2001; 2002). Creating energy demand by adrenalectomy imposes a new steady state condition characterised by profound activation of CRH and ACTH as if demanding the need for glucocorticoids that are not there. Surprisingly, the neuroendocrine activation is eliminated by providing the energy substrate sucrose, which the animals select to consume when offered together with saline. The brains of the sucrose-fed adrenalectomized rats adapt in behaviour (feeding, sucrose preference) and in HPA regulation. On the basis of this finding LAUGERO et al. (2001) propose that many of the corticosterone actions actually may be indirect and result from its metabolic action. Because the hormone restores energy metabolism the adrenalectomy-induced ACTH/CRH hyperactivity.is also reinstated in a permissive manner. In contrast, corticosterone chronically infused intracerebroventricularly (icv) in the brains of adrenalectomized animals mimics the effect of stress on the brain. Corticosterone icv enhances basal and stress-induced ACTH release and blocks the effect of sucrose infusion (LAUGERO et al. 2002) suggesting that the hormone has activated a feed-forward loop in the brain. These findings have triggered an intriguing twist in the debate on the good and bad effects of corticosterone in brain.

Cells. On the *cellular* level using the hippocampal slice two general principles were revealed (JOËLS and DE KLOET 1989; 1992; 1994; JOËLS 1994; 2001). First, the control exerted by MR and/or GR appeared to proceed in a U- shaped manner. Ion conductance and transmitter responses were maximal in the absence of corticosterone when no receptor is active and in the presence of very high supraphysiological concentrations of the steroid when both receptors are active. Intermediate corticosterone concentrations occupying predominantly MR and little GR that reflect the average steroid concentration during the day, do minimise the cell responses. For instance the 5HT1A-induced hyperpolarisations of CA1 and dentate gyrus neurons were minimal with MR stimulation, while they were maximal in tissue from ADX animals or from acute stimulation by high corticosterone concentrations. The magnitude of the latter 5HT1A receptor mediated response diminished if tissue was obtained from animals that had experienced prior to the experiment a prolonged period of chronic stress or excess corticosterone.

Second, these responses form the mechanistic basis for phenomena on the network level such as LTP, that also have been demonstrated to show a U-shaped dose responsiveness to corticosterone (DIAMOND et al. 1990). On a more generalised note the cellular work demonstrates that MR stabilises excitability on the cell and circuit level in hippocampus, while GR suppresses excitability transiently raised by excitatory stimuli. The design of the experiments also revealed the conditional nature of the effects. The resting membrane potential was not changed by corticosterone exposure and the steroid effects only became detectable after stim-

ulation by membrane signalisations. Thus the nature and context of these signalisations suggest an enormous diversity of corticosterone action in the control of cell homeostasis.

Neuroendocrine regulation. In neuroendocrine regulation the intracerebral MR and GR blockade using rather selective antagonists exerts a profound and differential effect on measures of HPA activity. In all experiments adrenally intact animals were used. The basis of our experiments was that we distinguished the blockade of GR in the HPA core (i.e. pituitary corticotrophs and PVN micro-environment) from blockade of MR/GR in stressor-specific afferents from brain stem, amygdala-locus coeruleus, prefrontal cortex and hippocampus. The latter blockade interferes with processing of information and behavioural responses with consequent changes in HPA regulation. Thus, exposure to a novel environment was used as stressor, since the limbic-cortical brain circuits involved in e.g. attention, appraisal, fear, and reward abundantly express MR and GR. The studies showed that the MR antagonist RU 28318 causes a rise in circadian basal trough and peak levels of HPA activity as well as an enhanced response to a novelty stressor (RATKA et al. 1989). This effect after central MR blockade was maintained after intrahippocampal administration (VAN HAARST et al. 1997). The GR antagonist mifepristone had no effect on basal trough activity as expected because no GR is occupied under these conditions. Rather GR blockade attenuated and prolonged the response to the novelty stressor (RATKA et al. 1989). The attenuation of the novelty-induced response was mimicked with antagonist application in the dorsal hippocampus, while the prolonged response required GR blockade in the PVN (DE KLOET et al. 1988; VAN HAARST et al. 1997, OITZL et al. 1995). Upon continuous infusion of a few ng icv of mifepristone the amplitude of the circadian rhythm became after four up to 14 days much more enhanced because the peak rather than trough levels in HPA activity did rise (VAN HAARST et al. 1996). This phenomenon very well could be an aspect of the beneficial therapeutic effect of mifepristone in psychotic depression. As is the case in the rat, chronic mifepristone enhanced reactivity of the flattened circadian rhythm in cortisol characteristic for the disease (BELANOFF, unpublished).

These experiments provide only a glimpse of what MR and GR activation in brain potentially can do to basal and stress-induced HPA activity. A number of points can be made in this respect.

First, there is a rich diversity in stressor-specific pathways activating the neurosecretory parvocellular CRH neurons of the PVN through predominant aminergic and GABA-ergic innervations (PALKOVITS 1999; COLE and SAWCHENKO 2002). These pathways include (I) the ascending brain stem aminergic inputs thought to mediate 'systemic' stressors as opposed to the 'processive' or 'psychological' stressors requiring processing of information in higher brain structures (HERMAN and CULLINAN 1997). (II) The input from the locus coeruleus-amygdala circuit that is important for the sympathetic tone. (III) The excitatory input of the left and right medial frontal cortex important for shifts of internal to external cues. (IV) The excitatory input from the hippocampus that depends on context. The aminergic pathways directly project to the CRH neurons, but many of the networks affect transsynaptically via an inhibitory GABA-ergic network the PVN. The excitatory inputs from limbic-cortical regions modulate the GABA-ergic interneuronal network in the PVN micro-environment (COLE and SAWCHENKO 2002; HERMAN et al. 2002) providing a stressor specific neurochemical signature to the neurosecretory neurons (PACAK and PALKOVITS 2001).

Second, tonic influences and feedback actions (DE KLOET and REUL 1987) exerted by corticosterone on the brain have an enormous diversity, since their rising levels feed back on the very same circuits that have initially led to their secretion. Thus corticosterone control depends on the phase of the CRH pulse generator (WINDLE et al. 1998), the nature and duration of the stimulus (LAUGERO et al. 2001) and mechanism involved in processing stressful information (KOVÁCS et al. 2000). AKANA et al. (2001) differentiated between frontal cortex and amygdala on the basis of metabolic and autonomic parameters from the perspective of stress-induced state dependent pathways. Our studies have addressed the hippocampus, because this structure plays an important

role in response to novelty and adaptation to stress. The inhibitory GABA-ergic network in the PVN micro-environment is a corticosterone target in its own right.

Third, the PVN is considered a dynamic entity integrating a diversity of signals that provide a neuroendocrine signature (ROMERO et al. 1996) to exert control over adrenocortical activation by neuroendocrine and autonomic pathways (BUIJS et al. 1999, HERMAN et al. 2002). In particular the stress-induced AP-1 pathway is blocked by corticosterone (KOVÁCS et al. 2000) suggesting a powerful vasopressin link in feedback regulations on the HPA core. At the same time by acting on the PVN corticosterone is capable to control the integrative function of the PVN both with respect to its afferent inputs as its central efferents controling neuroendocrine, autonomic and behavioural functions (DUAN et al. 1999). Corticosterone feedback is context-dependent and has an enormous diversity due to the great number of afferents stemming from peripheral (immune, metabolic, and inflammatory) and central sources. Given all these mutually interactive networks it will be a tall order to sort out the contribution of corticosterone to feedback in the HPA core *versus* each individual pathway under the great variety of stressor specific conditions.

Behaviour. Central MR activation stimulates autonomic outflow (VAN DEN BERG et al.1994; VAN DEN BUUSE et al. 2002), facilitates the conservation/withdrawal response if animals are exposed to a severe stressor (KORTE 2001) and enhances aggressive behaviour of a resident mouse to an intruder (HALLER et al. 2000). In the spatial learning tests MR affects interpretation of environmental information and selection of the appropriate behavioural response to deal with the stressor. Experimental evidence for this comes from the administration of a few ng mineralocorticoid antagonist icv immediately before testing, which altered the behavioural pattern in a maze in search for a route to escape or to find food that the animal had learned the previous day (OITZL et al. 1994; see figure 2). The neural mechanism underlying the latter MR-mediated action is not known and neither is known how the autonomous, neuroendocrine and behavioural consequences of central MR blockade mutually affect each other.

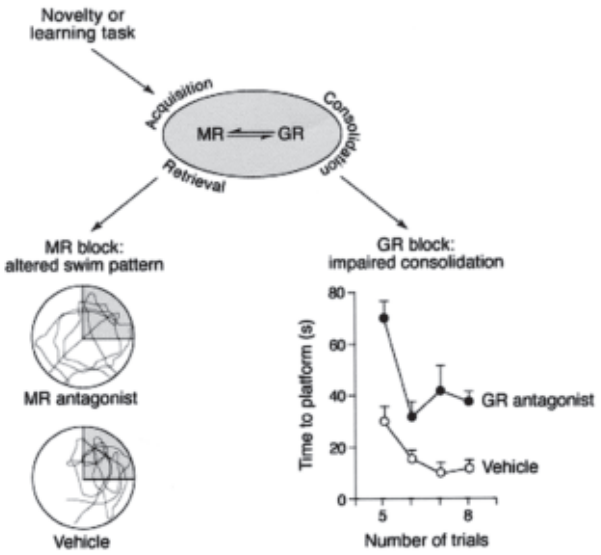

Fig 2 MR and GR affect different aspects of information processing. Following exposure to a learning task MR blockade, 30 to 45 minutes before retrieval on day 2, changed the swim pattern. Inhibition of GR immediately after acquisition on day 1 results in impaired performance 24 h later. These MR- and GR-mediated effects on information processing facilitate behavioural adaptation (from DE KLOET et al. 1999).

Blockade of brain GR impairs the storage of new information (Oitzl and de Kloet 1992; Sandi et al. 1997). A glucocorticoid antagonist administered around the time of learning in the hippocampus impaired the consolidation of newly acquired information As a consequence 24 hours later, the rat is unable to retrieve the information that was learned the previous day and has to learn the maze problem all over again. Likewise, mutant mice with a point mutation in GR, which obliterates binding to DNA, are unable to store learned information (Oitzl et al. 2001). This suggests that corticosteroid-induced cognitive performance requires transactivation as was previously found in the cellular responses to corticosterone in hippocampus (Karst et al. 2000) because such mutants lack the direct activation of GREs, but still have a GR that can interact with other transcription factors (Reichardt et al. 2000). Transgenic mice with downregulated GRs (knockdown) show also cognitive defects and elevated plasma ACTH and corticosterone concentrations in response to stress. After treatment with an antidepressant GRs are increased in concentration and simultaneous behavioural and hormonal corrections (Montkowski et al.1995). Mice exposed to chronic stress and high corticosterone deteriorate in spatial learning, while the reverse occurs after chronic treatment with GR antagonists which appears to result in enhancement of cognitive performance (Oitzl et al. 1998).

How do these MR- and GR-mediated effects on cognition relate to emotional behaviour? This can be illustrated in the following experiment measuring an anxiety paradigm. After exposure to a stressor activation of GR-dependent mechanisms promotes storage of newly acquired information (Korte 2001). This memory is helpful to predict the nature of upcoming events if the animal is exposed to the same place and context. During subsequent visits to the same "stressful" situation the individual's initial response is triggered by the previous experience. It depends on MR, because this behavioural repertoire is blocked by an MR-antagonist given just before behavioural testing suggesting anxiolytic activity of such antagonists. It is also blocked by exogenous GR antagonists prior to the initial stressful event the day before. One day later memory to the previous stressful experience is extinguished. Accordingly, blockade of brain GR interferes with cognitive aspects of fear and anxiety. This action likely takes place in the amygdala (Roosendaal 1999; McGaugh 2002), while the examples from spatial learning relate to the hippocampus (de Kloet et al. 1999).

MR and GR operate in two stress system modes

The key CNS systems generating the stress response have two modes of operation that involve two families of CRH-related peptides (Holsboer 2003; Hauger et al. 2003) (table 1). One mode in-

Table 1

STRESS	ADAPTATION
CRH	Stresscopin
CRH-1 receptor	CRH-2 receptor
sympathetic	para-sympathetic
immediate	late sustained
fight/flight	coping
MR	GR

CRH-1 and CRH-2 receptor systems drive the immediate response mode and the late adaptive mode of the stress system (see also Hsu and Hsueh 2001). MR determines the threshold or sensitivity of the fast response; GR represents the slow adaptive mode that terminates the fast response and that prepares for the future through storage of energy and information.

volves the fast, CRH-driven, neuroendocrine/sympathetic 'fight-flight' response mediated by CRH-1 receptors The fast responding system includes CRH-producing neurons located in the PVN, the amygdala, the noradrenergic neurons located in the locus coeruleus and other aminergic cells in the brain stem. In the periphery the adrenal cortex producing, amongst others, cortisol and the adrenal medulla secreting catecholamines, particularly adrenalin are the principal pacemakers.

The other slower system promotes recovery and adaptation and seems activated by the recently discovered urocortins acting via CRH-2 receptors (Hsu and Hsueh 2001; Reul and Holsboer, 2002). The urocortin II (stresscopin-related) and urocortin III (stresscopin) peptides have a distinctly different localisation from CRH and were identified as selective ligands for the CRH-2 receptor system (Hsu and Hsueh 2001; Lewis et al. 2001). Urocortin I is synthesised in a discrete region in the midbrain, the Eddinger Westphal nucleus, and binds to both CRH receptor sites. Urocortin II is expressed in PVN and locus coeruleus and urocortin III in the hypothalamic area rostral of the PVN, the preoptic nucleus and medial amygdala, but not in cerebellum, cerebral cortex or pituitary. Their terminal fields innervate hypothalamic and brain stem areas matching CRH-2 receptor distribution (Li et al. 2002).

Administration of the urocortins II and III evokes anxiolytic responses as opposed to the anxiogenic depression-like behaviour and hypersensitivity evoked by CRH (Hsu and Hsueh 2001). Some phenomena after CRH are also observed in animal models of depression (e.g. decreased food intake, inhibition of sexual behaviour, sleep disturbances and psychomotor activation). Opposing actions are being recorded for the urocortins II and III and that has led some (Hsu and Hsueh 2001) to suggest that CRH and urocortin are two anti-parallel stress systems that function as organisers of the sympathetic and parasympathetic response, respectively. These data strongly suggest a role for balanced CRH/urocortin family of peptides in the pathophysiology of states of anxiety and depression.

Synthesis. How are the corticosteroids implicated? The cellular data in various limbic regions suggest globally that MR prevents disturbance of homeostasis, while GR promotes its recovery. On the systems physiology and behavioural level this would imply that MR is implicated in a mechanism determining the threshold or sensitivity of the CRH - CRH-1 receptor-driven stress system response. Through GR the stress-induced activation in the various modalities of stress system afferents and in the hypothalamic-pituitary CRH/POMC core of the system are facilitated in termination. In this way GR is assumed to act synergistically to the late responding urocortin II/III - CRH-2 system promoting recovery and adaptation (Table 2).

Table 2

- MINERALOCORTICOID RECEPTORS (type 1)
  - prevent disturbance of cellular homeostasis
  - control sensitivity stress response systém
  - help to select behavioural response
- GLUCOCORTICOID RECEPTORS (type 2)
  - control energy metabolism
  - facilitate recovery of cellular homeostasis
  - restrain stress-induced responses
  - promote information storage
  - promote behavioural adaptation

Function of MR and GR on the cellular, neuroendocrine and behavioural level (see also de Kloet et al. 1998; 1999).

We postulate that the balance in these two stress systems is important for maintenance of health and homeostasis (DE KLOET et al. 1998). Figure 3 depicts this stress system balance idea in its stable (homeostasis) and labile (allostasis?) (MCEWEN and WINGFIELD 2003) version. It implies that in case of imbalance that the individual loses the ability to maintain homeostasis, if challenged by an adverse event. This may lead to a condition of neuroendocrine dysregulation and impaired behavioural adaptation as risk factor for the precipitation of depression (HOLSBOER 2001; 2001; 2003). It is in

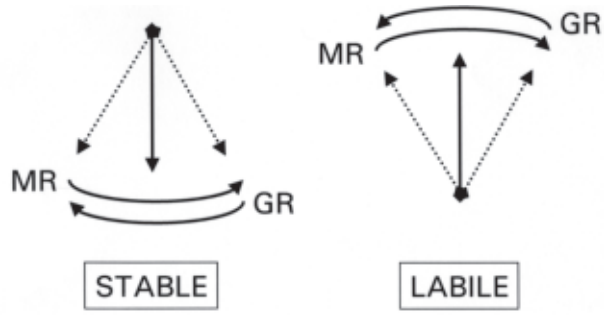

Fig 3 MR- and GR-mediated effects as indices for stress system activity. Figure depicts stable (homeostasis) and labile (allostasis?) representations.

this arena that the conversion of good vs. bad corticosteroid effect occurs. If coping with stress fails, corticosteroids fail to terminate the stress reactions and targets are exposed to elevated corticosteroid concentrations for a prolonged period of time. It is thought to sustain positive reverberating feedback loops (GOLD et al. 2002) that further aggravate the condition of imbalance. In the next sections I describe two animal models generated by 'nature-nurture' inputs that may be instrumental to dissect further the signalling pathways involved in stress system imbalance.

Animal models

Genetically selected mouse lines. Stress system responses display a large inter-individual variation in a normal population. In males the extremes display either an active fight/flight or a passive/conservation withdrawal response to a psychosocial challenge. Active animals rely on stable living conditions, show impaired adaptation to changing environment, display territorial aggression and flee after defeat. Their sympathetic response pattern dominates. Passive animals thrive better on changing conditions and they seem to be more dominated by parasympathetic activity and have high circulating cortisol levels after stress. Mouse and ratlines have been selected that represent these extremes in stress system activity (LANDGRAF et al. 2002; BOHUS et al. 1987).

In the research of the late professor Bohus the selection of male wild house mice for long and short latency before attack of the intruder in the home territory also did accumulate many of the traits characteristic for active and passive coping styles. The Short Attack Latency (SAL) mice display an innate active coping style towards environmental challenges with high stress-induced sympathetic and low adrenocortical activity. The Long Attack Latency (LAL) mice show a passive coping style and higher stress-induced corticosterone level. The SAL and LAL mice differ in many other parameters. For instance, the 5HT1A receptor expression and responsiveness in hippocampal CA1 neurons is more than 30% lower in LAL than in SAL (Veenema). Interestingly LANDGRAF et al. (2002) have selected rat lines based on emotional responses and these line differences could be eliminated with a V1A antagonist. This finding supports the evidence that in males vasopressin is also involved in genetic differences in anxiety and aggression e.g. fight or flight responses. In females oxytocin is more prominent in coping with a psychological stressor. Oxytocin promotes aspects of social behaviour, leading some to formulation of the tend-to-be-friend concept (CARTER 1998).

These extremes selected for aggressive and emotional behavioural traits may actually represent individuals in which either the CRH-1 or CRH-2 stress system modes dominates. In Veenema's studies basal hippocampal MR and GR, and hypothalamic CRH expression was not different. In subsequent experiments the LAL mice were repeatedly exposed to defeat or to the threat of defeat for 25 days. It appeared that the threat of defeat that is LAL's living next-door to the SAL only in sensory, but not physical, contact generated some of the features demonstrated in patients

suffering from depression notably an enhanced adrenocortical output and a lower MR/GR ratio (VEENEMA et al. 2003; VEENEMA, this conference). Hence, the passive behavioural coping style combined with low hippocampal 5HT1A receptor function and elevated circulating corticosterone levels predicts to some extent enhanced stressor susceptibility. These measures if further validated may match in males the criteria for an animal model for depression. Whether the same reasoning holds for female mice remains to be seen, because of their entirely different coping style, i.e. the formation of female-female social bonds involving estrogens and oxytocin rather than the sympathetic fight-flight response of males that was the basis for the SAL vs LAL selection.

Early life trauma. Individual differences in stress system activity are not only determined by genotype, but also by cognitive and non-cognitive (e.g. metabolic, immune) inputs, and experience related factors (LEVINE et al. 2000) Rearing experiences are in animals particularly potent and may permanently alter behavioural and endocrine stress responses. For instance, handling of rats (i.e. daily separation for 15 min from the mother) provides a brief intermezzo in mother-pup interaction, which subsequently results in increased sensory stimulation by intensified maternal care. As adults, the neonatally handled animals exhibit reduced fearfulness more rapid activation and termination of the adrenocortical responses than their non-handled littermates, while spatial learning is improved. These permanent effects on the stress system and cognitive performance seem to be associated with increased GR number, increased synaptogenesis and increased expression of BDNF mRNA in hippocampus (LIU et al. 2000). These findings have led to the concept that variations in maternal care form the basis for stable inter-individual differences in later stress reactivity and cognition through changes in gene expression patterns in the stress system (LEVINE et al. 2000, MEANEY 2001). Maternal care also affects the maternal behaviour of female offspring. Maternal behaviour is dependent on estrogen-oxytocin interactions, which form the basis of intergenerational transmission of individual differences in stress reactivity (MEANEY 2001).

In contrast, early adversity can lead to an anxious animal, more prone to develop depression-like behaviour if stressed, particularly so if the animal is genetically 'primed'. Adult rats that had been subjected to daily 6-hour separation from their mother during postnatal days 2-20 showed greater stress-induced increase of plasma ACTH and corticosterone levels, than rats raised under normal conditions. Moreover, CRH concentrations in CSF and in the median eminence were elevated, expression of CRH mRNA in the PVN was increased, CRH binding in the pituitary decreased and that in the extrahypothalamic CRH system increased (ARBORELIUS et al. 1999). CRH and noradrenergic drive are obviously increased, and the GABA / benzodiazepine "tone" decreased. Behaviourally these animals are anxious, show decreased social interaction and impaired cognitive functioning (KAUFMAN et al. 2000).

We have examined the life long effect of a single maternal deprivation. Infant rats of the Brown Norway strain were maternally deprived for 24 hours at postnatal day 3. Spatial learning ability in the Morris water maze and circulating corticosterone was measured at 3, 12, 24 and 32 months of age. The results show that until midlife the cognitive performance of rats deprived as pups declined faster in the face of a dramatically enhanced HPA responsiveness to stress than in the mother reared control animals. During aging this difference in cognition and HPA responsiveness between deprived and control animals vanished, but instead the deprived animals showed a strongly enhanced individual variation in performance (figure 4). Thus, the majority of the mother-reared senescent animals were partially impaired with few animals either unimpaired or fully impaired. In contrast, maternal deprivation drives spatial learning ability to the extremes, at the expense of the average. This implies that in the deprived senescent group there are almost no partially impaired animals. About 50% is impaired and 40% shows excellent performance (OITZL et al. 2000). The latter "successful agers" have the highest expression of brain-derived neurotrophic factor (BDNF) in the hippocampus (SCHAAF et al. 2001) The high expression of BDNF in the cognitively unimpaired senescent animals supports the con-

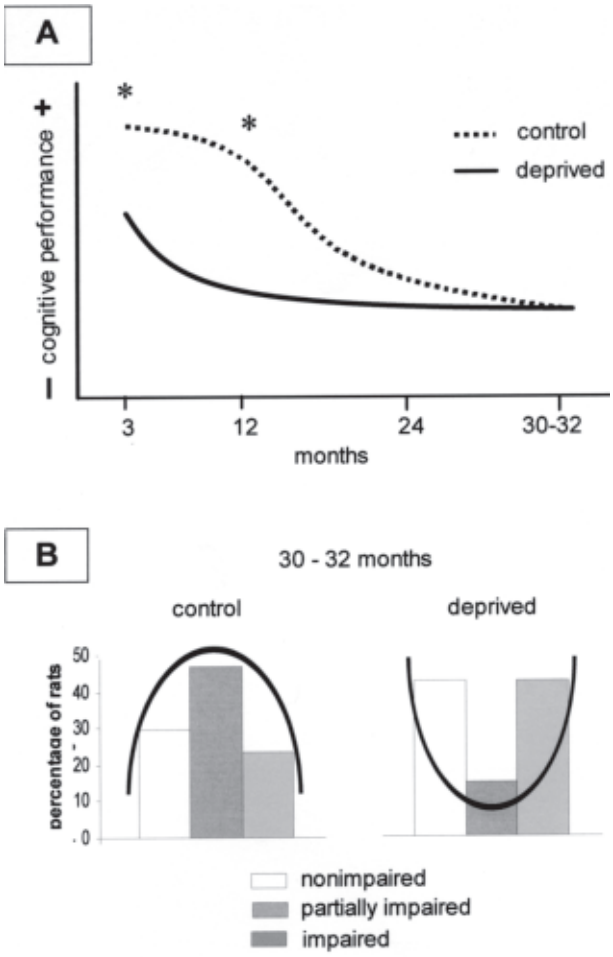

Fig 4 Cognitive performance of male Brown Norway rats deprived as pups from maternal care.
A**: Rats were maternally deprived at post natal day 3 for 24 hours (deprived) or were maternally reared (controls). Note the impairment of performance in the spatial learning test at 3 and 24 months of age. At senescence control and deprived rats are partially impaired and in average not different.**
B**: Maternally deprived rats display during aging enhanced individual variation in performance. The majority of the controls is partially impaired. In the deprived animals aging drives cognitive performance to the extreme; the senescent rats are either impaired or not impaired, at the expense of the average partially impaired performance (from** OITZL **et al. 2001).**

cept that this growth factor is implicated in the regulation of synaptic plasticity underlying memory performance.

The results show that healthy senescent Brown Norway rats do not have elevated corticosterone levels, but rather that corticosterone levels are lower than in the controls (WORKEL et al. 2001; VAN EEKELEN et al. 1995). These low levels at senescence were attained after that the maternally deprived rats had at mid-age (12 months) a period of strongly enhanced adrenocortical activation in response to a novelty stressor. In other rat strains some aspects of cognitive decline are linked to elevated corticosterone levels in a subgroup of rodents. Long-term amitryptiline treatment from midlife onwards improves cognition, decreases indices for anxiety and restores HPA activity in this subgroup (YAU et al. 2002). Collectively the data suggest that adrenocortical activity at midlife determine a trajectory of aging which can be influenced by pharmacological intervention. This line of reasoning finds support from a recent study of LUPIEN et al. (1999) showing that memory function of the elderly can be intensely modified by pharmacological treatment with glucocorticoids, although the direction of the effects depends on the cortisol history of each individual. When aged individuals are treated with metyrapone their resulting memory deficits were corrected with cortisol if they had a 5-year history of moderate cortisol levels. If cortisol levels had been high over that same period cortisol administered to the metyrapone-treated elderly further deteriorated cognitive function.

In conclusion, in rodent pups maternal care programs for life inter-individual differences in adrenocortical and emotional reactivity, and in cognitive performance. These individual differences are amplified at senescence in case of deprivation of maternal care. In this trajectory of aging that leads to a more clear-cut dissociation between good and bad performers, the history of cortisol exposure seems to be an important determinant. Deprivation from maternal care is a laboratory model of neglect, which can be taken as model for abuse. The outcome of this type of early trauma depends on the gender and strain (genetic background) of the pups as well as the time point and the duration the pup is deprived from maternal care.

Future directions

This essay is based on the thesis that the stress system operates in an immediate fast responding and a slower adaptive mode in which the balance

in brain corticosteroid receptor-mediated actions is one of the control nodes. This balance could be viewed as the set-point of the stress system in maintaining stabile or labile equilibrium in life processes (figure 5), a discussion which is presently gaining momentum (McEwen and Wingfield 2003). Selye advocated the opposing actions of mineralocorticoid and glucocorticoid hormones and took as criterion their pro- and anti-inflammatory effects respectively. Our work has given a central position to MR- and GR-mediating the action of one single hormone: corticosterone. Collectively, the data suggest that MR-mediated actions are directed to maintain equilibrium and health, while GR promotes their recovery.

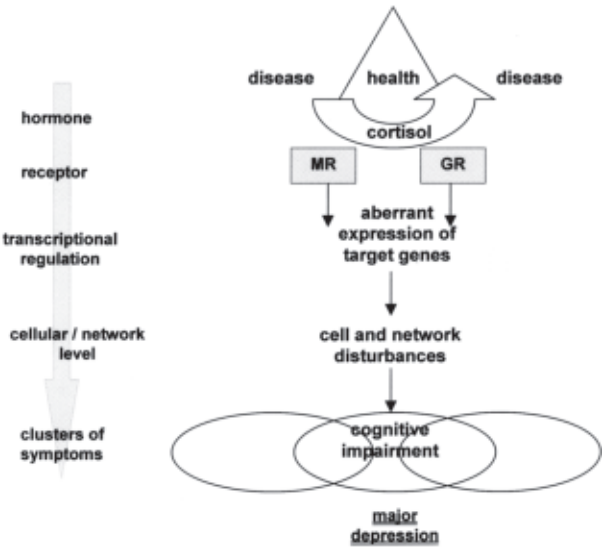

Fig 5 The MR – GR balance hypothesis.
The balance in MR – GR-mediated effects is thought crucial for homeostasis and health. Imbalance either by excess MR or excess GR stimulation is proposed to enhance vulnerability to disease the individual is predisposed. The scheme depicts the various levels of analysis.

Evidence was shown that on different levels of biological complexity the properties and localisation of MR and GR direct the type of molecular changes and cellular/network properties that underlie behavioural adaptation. Stress system imbalance induced by chronic stressors compromises these processes from gene to behaviour and increases disease vulnerability for which the individual is predisposed. Thus aberrant corticosteroid concentrations cause altered function and structure of discrete brain areas affecting such fundamental processes as apoptosis, neurogenesis and synaptic plasticity (McEwen 1999; 2002; Sapolsky 2000; Czeh et al 2001; de Kloet et al. 1999).

The challenge today is to combine knowledge on the function of individual gene products identified by genomic screening with holistic approaches in the analysis of higher brain functions i.e. emotions and cognitive processes. Technical innovations, such as the application of *in vivo* si-RNA technology and candidate gene single nucleotide polymorphism (SNP) analysis will greatly facilitate progress in the molecular arena. On the other hand neuro-imaging technology (Drevets et al. 1997) will help to pinpoint responsive brain circuitry in patients that have been diagnosed with greater precision using neuropsychological and systems physiology approaches. I see new developments in many areas, but would like to mention three aspects with particular bearing for the field of stress hormones.

Genetics of the corticosteroid receptor system

I expect that the coming years SNP's will be identified in the corticosteroid - MR/GR transcription machinery that bias corticosteroid control of the stress response. The 11-HSD defect providing local excess of cortisol resulting in metabole syndrome is an excellent example (Masuzaki et al. 2001). Likewise one could predict that certain SNP's may bias aspects of receptor signalling which may cause local imbalances in homeostatic control (de Rijk et al. 2001; van Rossum et al. 2002).

Genomic screening

The initial observations have opened up a bewildering array of stress-responsive genes (Datson et al. 2001; Feldker et al. 2003) that need to be analysed to answer questions such as how, where and when these genes become active in stress-induced signalling pathways, and foremost what their precise function is (Sabban and Kvetňanský 2001). Recently, in a hippocampal transcriptome both SAGE and GeneChip analysis showed under

basal conditions a higher expression of several cytoskeleton genes in LAL (the passive copers) hippocampi than in SAL (active copers), as well as higher expression of a number of calmodulin-related genes and genes encoding components of a MAPK-cascade. Accordingly, this differential regulation of a raf/ERK pathway may be related to structural differences in hippocampus of LAL and SAL mice that can be taken marker for *predisposition*. A chronic psychosocial stressor produced a phenotype characterised by a dysregulated stress system. In the hippocampus down regulation of CHUK a kinase involved in the regulation of NFkB was observed, as well as down regulation of several Ras oncogene family members. These genes may be considered a marker for stress-induced change or a molecular marker for e.g. *pathogenesis* (FELDKER et al., in press).

Animal models

Behavioural tasks in which the analysis of simultaneous emotional and cognitive processes is combined with neurophysiological network analysis may lead to better animal models. This may open up questions on the mode of action of stress hormones in control of cognitive processes leading to the precipitation of emotional disturbances characteristic of for example depression. A distinction should be made in this respect between (i) the core of the HPA axis with emphasis on dysregulations in the PVN microenvironment in its organization of the stress response and (ii) dysregulations in specific afferent stress circuits to the PVN e.g. medial prefrontal cortex, hippocampus, amygdala and brain stem, that are targets for the stress hormones. This is important because it becomes increasingly clear that a novel generation of drugs may arise from targeting pathway nodes in stress regulation centers in the brain to treat stress-related brain disorders and co-morbid medical consequences such as metabolic, cardiovascular and neurodegenerative diseases. The potential success of the GR antagonists in the treatment of severe depression (BELANOFF et al. 2002) hints that the therapeutic focus on stress circuitry may be a rewarding approach.

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