Numerous studies employing immediate-early gene expression or functional magnetic resonance imaging have provided evidence of the neural networks which are activated by different stress modalities and which serve to coordinate the different elements of the stress response. For example, as expected from the activation of the hypothalamo-pituitary-adrenal axis, c-fos mRNA or its protein product Fos can be detected in the hypothalamic paraventricular nucleus in response to both psychological (exteroceptive) and physical (introceptive) stress stimuli. However, these two modalities are differentiated by their activation of limbic and brainstem nuclei, notably areas of the amygdala and bed nuclei of the stria terminalis display marked modality-dependent activation. Nevertheless, while different stress modalities may utilize distinct neural pathways which converge on common outputs, each may be generalised to comprise two elements. Firstly, an afferent limb comprising either a cognitive or sensory system that responds to introceptive or exteroceptive stimuli which under certain conditions or above a particular threshold can take on a 'stressful' quality. Secondly, an efferent limb comprising a network which distributes this 'stress signal' to the various effector systems to generate an appropriate physiological or behavioural response. Diversity of stress responses and integration within this network is proposed to be achieved through a hierarchy of overlapping pathways, with some having the capacity for direct, rapid activation in response to an immediate threat to homeostasis, while higher order pathways provide a distributed signal to several output systems. It serves little purpose to identify the neurochemical phenotype of all the neurones which contribute either to the afferent or efferent limbs of these stress-activated networks. However, certain transmitters have been shown to have a pre-eminent role in regulating stress response, either acting to coordinate activation of diverse elements of the response (notable corticotropin-releasing hormone), or to attenuate activation of the response network and, thereby, have properties of endogenous anti-stress agents (e.g. oxytocin or GABA). It is suggested that the organisation of the response-activating network enables these key transmitters to fulfil important roles in integrating the stress response or in modulating its magnitude. Both classes of transmitter are particularly important as they may play important roles in the aetiology of stress-related disorders and, consequently have become the focus for pharmacological intervention. Furthermore, the involvement of transmitter receptor subtypes opens the possibility for the development of selective ligands for modulating the diverse responses to stress. In this respect an on-going challenge is to translate the knowledge of transmitter involvement into clinically effective therapies.

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SA9

Dissecting glucocorticoid receptor actions in stress by targeted mutations in mice

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Glucocorticoids acting through the type II glucocorticoid receptor (GR) are critical for maintenance of homeostasis after both psychological and physiological stress. This notion is exemplified by impaired survival of humans with adrenal insufficiency, the association of dysregulation of the hypothalamic-pituitary-adrenal axis with psychiatric disorders, and the bidirectional interplay of the adrenal axis with the immune system. To understand the essential actions of glucocorticoids acting through GR to maintain homeostasis, identification of key cell and gene targets is needed. In this regard, genetic model systems to define mechanisms of GR actions at the cellular and genomic levels have proven informative. Global deletion of GR results in neonatal lethality and dramatic glucocorticoid overproduction, a phenotype that precludes analysis of GR function in later development and that may confound interpretation by grossly elevated glucocorticoid concentrations exerting effects on other nuclear receptor types. To minimize these limitations, we have utilized the Cre recombinase – loxP system to delete GR specifically in the forebrain, T cells, and the macrophage/neutrophil lineage. Mice with forebrain-restricted deletion of GR (FBGRKO) demonstrate heightened adrenal axis activity, despair-like behaviours responsive to anti-depressants, and aberrant locomotor activation in association with stress. Given the adult onset of deletion of GR in FBGRKO mice and the temporal relationship of GR deletion to behavioural abnormalities, these effects are not likely due to developmental aspects of GR function but rather to changes in glucocorticoid responsiveness in the adult. Mice with deletion of GR in T cells (TGRKO) or macrophages (MGRKO) demonstrate augmented cytokine production and increased lethality with immune system activation. Cyclooxygenase (COX)-2 proved to be a critical target for GR actions as COX-2 inhibitors rescued each line from lethality after immune activation. Our recent studies in MGRKO mice have identified GR modulation of p38 MAPK activity as a key target for GR anti-inflammatory actions after toll-like receptor 4 engagement, while ERK, Akt, and JNK were not glucocorticorticoid responsive. Our ongoing studies will attempt to more rigorously restrict GR deletion to specific brain and body sites utilizing a lentivirus-Cre delivery system to facilitate later gene profiling efforts.

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SA10

Mineralocorticoid and glucocorticoid receptors in the stress response

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The balanced interaction of the various humoral and neural mediators that serve to contain stress reactions in the acute phase and in the management of the late recovery phase is of crucial importance for defense of homeostasis and health. Imbalance due to inadequate or excessive operation of these stress system mediators may compromise resilience and promote a phenotype vulnerable to stress-related disease. The importance of a 'balance' in the concentration and action of stress mediators is illustrated by the glucocorticoid hormone (cortisol or corticosterone). The hormone operates both in the *fast* and *slow* modes

of the response to stress and produces lasting effects in preparation of future events. The two complementary modes of operation of cortisol/corticosterone depend on the phase and context of the stress response, the bio-availability of the hormone and the target cell response. The molecular basis of this dual mode of operation of cortisol is formed by two types of receptors - mineralocorticoid (MR) and glucocorticoid (GR) receptors - that bind *in vivo* cortisol and corticosterone with an order of magnitude difference in affinity. Thus, a concept has evolved in which MR and GR mediate the dual mode of operation of cortisol in limbic brain to coordinate the *onset* and *termination* of the physiological and behavioural adaptations to the stressor. MR and GR belong to interacting signalling networks that underlie adaptive processes from appraisal of novel situations and prediction of upcoming events to recovery from the challenge and storage of the experience in the memory. Thus questions to be addressed are: which factors determine balanced MR/GR interaction? How is MR/GR imbalance related to a vulnerable phenotype? How does the MR/GR balance contribute to individual differences in vulnerability? Which biomarkers can reveal imbalance in stress system operation related to MR and GR?

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SA11

Stress, chromatin remodelling and behavioural adaptation

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Coping with stressful events is part of everyone's daily life. It is thought that changes in gene expression are involved in the neuroplasticity processes underlying stress coping. Gene expression is controlled by transcription factors whose activity is governed by a variety of signal transduction cascades. Control of gene expression is tight and normally part of the genome is silent with the nucleosomes structurally organized in condensed chromatin. The nucleosomes consist of highly organized complexes of DNA and histone molecules and in condensed chromatin they are inaccessible for transcription factors. Nuclear receptors such as the glucocorticoid receptor (GR) are an exception to this rule as they are able to access their hormone responsive elements and 'unlock' the nucleosome rendering it accessible for molecules involved in chromatin remodelling and gene transcription (1). Recently, the concept has arisen that distinct post-translational modifications in the N-terminal tails of histone molecules play a decisive role in chromatin remodelling. The phosphorylation of histone H3 at Ser10 and its acetylation at Lys14 (i.e. P(Ser10)-Ac(Lys14)-H3) have been associated with the local opening of condensed chromatin allowing the transcriptional activation of dormant genes (2). It appears that the Ser10 residue in histone H3 comprises a converging point of multiple signal transduction pathways and kinases but these have been hardly investigated *in vivo*.

Recently, we demonstrated for the first time that psychologically stressful stimuli such as forced swimming, predator exposure and novelty increase the phospho-acetylation of histone H3 in dentate gyrus granule neurons of rats and mice (3; Y. Chandramohan, SK Droste & JMHM Reul, unpublished observations). The nuclei of these neurons showed a speckled staining pattern which has been shown to be associated with transcriptional activation (e.g. (4)). The enhanced histone H3 phospho-acetylation was neuroanatomically quite specific because the stress-induced increase in P(Ser10)-Ac(Lys14)H3-positive neurons was only observed in mature (NeuN-positive) neurons in the middle and superficial aspects of the granular cell layer of the dorsal blade of the dentate gyrus (3; Y. Chandramohan, S.K. Droste & J.M.H.M. Reul, unpublished observations; Y. Chandramohan, S.K. Droste, J.S. Arthur, J.M.H.M. Reul, unpublished observations), suggesting that only mature neurons of a particular part of the dentate gyrus are recruited in the response to stress.

Follow-up studies revealed that stress-induced histone H3 phospho-acetylation is mediated by both glucocorticoid receptor (GR) and NMDA receptor (NMDA-R) signalling suggesting an integration of these two signalling pathways (3; Y. Chandramohan, S.K. Droste & J.M.H.M. Reul, unpublished observations). Blockade of the mineralocorticoid receptor (MR) was ineffective (Y. Chandramohan, S.K. Droste & J.M.H.M. Reul, unpublished observations). Furthermore, the forced swimming-induced increases in dentate histone modifications could be blocked by inhibiting the MAPK-ERK (mitogen-activated protein kinase–extracellular signal-regulated kinase) pathway and by genetic deletion of the mitogen- and stress-induced kinases 1 and 2 (MSK1/2; Y. Chandramohan et al. unpublished observations). Thus, the stress-evoked histone H3 modifications were mediated by GR signalling as well as signalling through the NMDA-R/MAPK/ERK/MSK1/2 pathway (3; Y. Chandramohan, S.K. Droste & J.M.H.M. Reul, unpublished observations). If rats or mice are subjected a second time to forced swimming 24 h after the first forced swim session, they attain an immobile posture in the water for about 70% of the 5 min re-test time. This is a stress-related cognitive response as the animal has learned from the first forced swim session that escape from the water is impossible. We observed that blockade of GR signalling or NMDA-R/MAPK/ERK/MSK signalling, but not MR signalling, resulted in a strong impairment of the behavioural immobility response (Chandramohan et al. unpublished observations).

Therefore, based on the strict correlation between the biochemical and behavioural responses, we have postulated that the histone H3 phospho-acetylation response after the initial forced swim session is required for the acquisition of the behavioural immobility response observed in the re-test. The histone modification response occurred in a distinct population of dentate gyrus granule neurons and required recruitment of both the GR and the NMDA/MAPK/ERK/MSK signalling pathways.

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SA12

Stress, cognitive function and cell adhesion molecules

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Stress is a potent modulator of brain and cognitive function. Depending on the circumstances, stress can either facilitate or impair memory processes. Our research aims to unravel how neurobiological mechanisms related to learning and plasticity are affected by stress conditions leading to opposite effects on memory function (facilitating vs. impairing). By focusing on key plasticity-related proteins, we investigate the mechanisms that translate stress' actions into such diverse cognitive outcomes. Our work has implicated the neural cell adhesion molecules of the immunoglobulin superfamily, NCAM and L1, on the effects of stress on brain and cognitive function (Sandi, 2004; Sandi & Touyarot, 2006). In the hippocampus, memory-facilitating and memory-impairing stress conditions lead to opposite patterns of expression of cell adhesion molecules (Sandi et al. 2005; Venero et al. 2006), which underscore these molecules as potential mediators of the cognitive effects of stress. Although glucocorticoid treatments do not reproduce exactly the changes on plasticity-related proteins induced by stress, available evidence indicates that these steroid hormones play a major role on stress-induced modulation of both cell adhesion molecules, and other plasticity-related proteins. These findings (1) have implications to understand stress in the context of both pathology and adaptation, and (2) underscore cell adhesion molecules as potential therapeutic targets in stress-related cognitive disturbances (Cambon et al. 2004).

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SA13

Dendritic and synaptic remodelling in mammalian hippocampus following stress

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Chronic restraint stress (CRS), in which rats are held for periods of 6 h/day for up to 21 days, induces raised corticosteroid levels and may result in cognitive impairment. It causes a variety of morphological changes in hippocampal areas, including loss of dendritic arborisation but, at least in area CA3, no significant neuronal loss. We have examined the effects of 21 days of CRS on the ultrastructure of synapses, dendrites and spines in rat hippocampal areas CA3 and CA1. In order to ensure that our data were not biased by volume changes the Cavalieri method was used to assess the effects of CRS on hippocampal volume. Following CRS, the mean total hippocampal volume decreased by ~15%, and for dorsal CA1, the volume was reduced by ~16%; in contrast, in CA3 there were no significant volume differences between control and stressed rats. These data were used to correct density measurements in subsequent studies.

At the ultrastructural level, using unbiased 2-D stereology, we found that there is a loss of simple unperforated synapses in striatum lucidum of CA3, which can be reversed rapidly by spatial learning (Sandi et al. 2003). To determine the real nature of synaptic and dendritic changes we used full 3-dimensional reconstruction (software from Fiala and Harris: http://synapses.bu.edu) of these structures (Stewart et al. 2005). Up to 150 serial sections (~50-70nm in thickness) were taken for each of the 3-D reconstructions of dendritic segments (~20 µm in length) of CA3, to show whole thorny excrescences (the spine complexes found in CA3), and postsynaptic densities (PSDs) on the thorns, for each of four functional animal states: (i) unrestrained controls, (ii) restrained, (iii) water maze-trained and (iv) water maze-trained, following restrain stress. A segment of dendrite from CA3 with thorny excrescence is shown in Fig. 1. Note the thorns with post-synaptic densities (PSD) in red, and also a single mushroom spine, also with a PSD (scale bar, 5µm). Following 21 days of chronic restraint stress (CRS), our 3-D data show that in CA3 there is a decrease in volume of thorns and a retraction of thorns in CRS compared with unrestrained rats. However, there is no change in the number of thorns per thorny excrescence. CRS induces a decrease in both endosome content and coated vesicles in thorns, processes which are substantially reversed 24 h following water maze training and indicating most probably in relation to the endosome changes, altered receptor recycling. However, in CA1 changes in 3-D structure are not so marked (Donohue et al. 2006). Our data from CA1 (so far only with stressed and control rats), have shown no quantitative changes in spine parameters between groups but significant increases in PSD membrane surface area (>36%) and in PSD volume (>60%) in stratum lacunosum moleculare of the CRS group. A highly significant overall increase in the 'PSD surface area/spine surface area' ratio also occurs in the CRS group (>27%).

Overall these data indicate that as a result of CRS there is a highly selective structural remodelling of dendritic and synaptic contacts within CA3 and CA1, which for CA3 can be rapidly reversed by a behavioural training task, demonstrating the remarkable neuroanatomical plasticity of CA3.

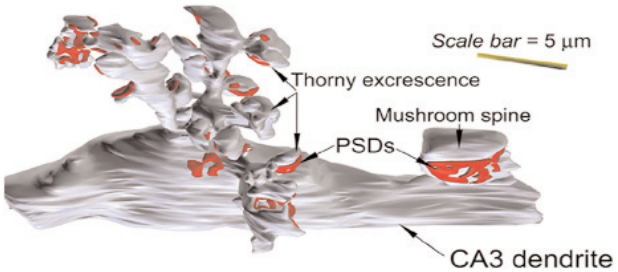

Figure 1. A 3-dimensional reconstruction from 150 serial ultrathin sections of a 20 µm segment of dendrite from CA3 with thorn excrescences. Note that the thorns show post-synaptic densities (PSD) in red, and there is also a single mushroom spine, with a PSD (scale bar, 5µm).

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SA14

Glucocorticoids and perinatal programming

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Epidemiological evidence suggests that an adverse fetal environment permanently 'programmes' physiology leading to increased risks of cardiovascular, metabolic, neuroendocrine and psychiatric disorders in adulthood. In a variety of animal models, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the feto-placental 'barrier' to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic-pituitary-adrenal (HPA) axis activity and anxiety-related behaviours in the adult offspring.
In humans, 11β-HSD2 gene mutations lower birth weight and placental 11β-HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Maternal glucocorticoid therapy alters offspring cognition and affect. Pregnant women exposed to the World Trade Centre atrocity appeared to transmit the neuroendocrine change to their 1-year-old offspring, predominantly if exposed in the third trimester.
The molecular mechanisms may reflect permanent changes in the expression of specific transcription factors, perhaps key is the glucocorticoid receptor (GR) itself. Differential programming of GR in different tissues, including hippocampus and amygdala, reflects effects upon one or more of the multiple tissue-specific alternate first exons/promoters of the GR gene. There are exquisitely targeted promoter-specific, and indeed transcription-factor binding site-specific, changes in DNA methylation that occur only during specfic sensitive periods of development. Curiously, some of these effects appear to be 'inherited' transgenerationally, affecting a further generation, itself unexposed to exogenous glucocorticoids at any point in the lifespan. Such effects can follow the male line, indicating epigenetic changes that persist through meiosis, fertilization and embryogenesis. Thus developmental exposure to excess glucocorticoids 'programmes' peripheral and CNS functions in adult life that may predispose to pathology and these effects may be transmitted into one or perhaps more subsequent generations.

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SA15

Stress and obesity: the evolutionary roles of glucocorticoids gone awry in our cultures

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Uncontrollable chronic stressors include periods of drought, earthquake, hurricane and famine which demand searching for new territory and the drive and energy to support the search. Glucocorticoids (GC), secreted during stress, appear to adapt the organism perfectly to find a new, more hospitable site to live while maintaining metabolic energy for the search. In the brain, GC act to increase stimulus salience or motivation; the valence of the behaviour emitted depends on the conditions and state of the animal and available outlets. In rats, GC facilitate search and running behaviours, freezing, aggression, anxiety- and fear-like behaviours; they also stimulate ingestion of palatable fat and sugar, but not plain (boring) chow [1-4]. However, GC do stimulate chow intake in diabetic rats in a dose-related fashion, but insulin, acting through the hepatic vagus, stimulates lard ingestion while decreasing chow intake in diabetic rats [5-7]. In the periphery, GC are catabolic and mobilize substrates for hepatic gluconeogenesis, but they also stimulate insulin secretion, which, in turn determines which foods will be eaten. Together these hormones shift caloric stores from the periphery to central fat depots. However, there is a metabolic feedback signal to the hypothalamic-pituitary-adrenal axis as well as the well-known acute GC-mediated feedback at hypothalamus and pituitary. Central fat mass is inversely related to the magnitude of hypothalamic corticotropin-releasing factor expression [4], and voluntary lard ingestion by rats markedly reduces the amplitude of ACTH and corticosterone responses to acute restraint [7], suggesting that stressor-induced eating may serve as self-medication for protection against the central effects of stress. In current civilizations, where perceived stressors abound and palatable foods are readily available with minimum exertion, this evolutionarily brilliant set of actions of stress-induced GC almost certainly contributes to the current epidemic of obesity and the pathophysiologic association between abdominal obesity and the metabolic syndrome.

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