SA1

The MAPK pathway and Egr-1 mediate stress-related behavioural effects of glucocorticoids

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Activation of the glucocorticoid receptor (GR) by stress-induced high levels of glucocorticoids mediates many of the behavioural consequences of stress. To explore the molecular mechanisms of these effects, we combined *in vivo* and *in vitro* approaches. We analysed brain-specific GR mutant mice (GRNesCre) and cell lines that either express endogenous GRs or in which a constitutively active form of the GR is transiently induced. In cell lines and in the hippocampus after stress, GR activation profoundly increased the expression levels and the enzymatic activity of components of the MAPK pathway and led to an increase in the expression of Egr-1. In parallel, inhibition of the MAPK signalling pathway within the hippocampus abolished the increase in contextual fear-conditioning induced by glucocorticoids. The present results provide a molecular mechanism for the stress-related effects of glucocorticoids on fear memories.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA2

Glucocorticoid-NFkB interactions

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Glucocorticoids inhibit inflammation by acting through the glucocorticoid receptor (GR) and powerfully repressing nuclear factor-kB (NF-kB) function (1). Ligand binding to the C-terminal of GR promotes GR nuclear translocation and binding to NF-kB through the GR DNA binding domain. NFkB is a multiprotein family of transcription factors, activated by proinflammatory cytokines. Family members all contain a conserved Rel domain, which binds DNA. Importantly, transcriptome profiling experiments have shown that expression of the p65 component of NFkB predicts the glucocorticoid response of bronchial asthma, a major human inflammatory disease widely treated with synthetic glucocorticoid drugs (2). Expression of NFkB p65 in cells impairs GR function. To identify how GR ligand binding influences the functional interaction between NF-kB and GR, studies were performed in human cell line models.

Both dexamethasone (agonist), and RU486 (antagonist) promote efficient nuclear translocation of the GR, and we show occupancy of the same intranuclear compartment as NF-kB with both ligands. However, unlike dexamethasone, RU486 had negligible activity to inhibit NF-kB transactivation. This failure may stem from altered co-factor recruitment, or altered interaction between the GR and NF-kB.

Using a GST pull-down approach we found that RU486 appeared to disrupt the GR-NFkB interaction. Following this bioluminescence resonance energy transfer (BRET) analysis identified a major glucocorticoid ligand effect on interaction between the GR and the p65 component of NF-kB, with RU486 inhibiting recruitment compared with dexamethasone. Using the BRET assay, we found that RU486 efficiently recruited NCoR to the GR, unlike dexamethasone, which recruited SRC1. Therefore RU486 promotes differential protein recruitment to both the C terminal (NCoR and SRC1) and DNA binding domain of the receptor (NFkB).

By disrupting the GR N-terminal, which contains the major transactivation domain AF-1, we were able to unmask significant transrepressive activity of RU486 on NFkB. Chromatin immunoprecipitation studies showed efficient recruitment of NFkB p65 to the NFkB response element on the interleukin 8 gene in response to TNF treatment, as expected. Also as expected, glucocorticoid treatment promoted recruitment of GR to the same element, but only under conditions that allowed prior recruitment of NFkB. Importantly, we show that impaired interaction between GR and p65 with RU486 leads to reduced recruitment of the GR to the NF-kB responsive region of the IL-8 promoter, again in contrast to dexamethasone (1). We demonstrate that ligand-induced conformation of the GR C-terminal has profound effects on the functional surface generated by the DNA binding domain of the GR. This has implications for understanding ligand-dependent interdomain communication.

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SA3

The glucocorticoid receptor and its variants

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Glucocorticoids exert a multiplicity of effects, yet all these effects appear to be mediated by the product of a single gene - the glucocorticoid receptor (GR). As the human genome has proved to consist of fewer genes than expected, attention has turned to variant forms of gene products as a source of increased proteome size and complexity of regulatory signalling networks.

Expression of the human GR is regulated by at least three distinct promoters (1A, 1B and 1C) that regulate transcription of mRNA species incorporating different exon 1 sequences. Promoters 1B and 1C regulate expression of mRNA species that are detected in most tissues, while promoter 1A functions selectively in haematopoietic cells. Alternative splicing of GR exons is known to produce several variants, although exon 1 is non-coding so that the different promoters do not encode distinct proteins. Alternative splicing of the eight coding exons is well established, however. The most extensively studied splice variant, GR beta,

results from usage of a different exon 9 to that used in the `classic' (GR alpha) form (1). The GR beta variant, which cannot bind ligand, may exert a dominant-negative effect on glucocorticoid signalling and a number of reports suggest that aberrant expression is linked to disease. Controversy continues to surround the likely significance of this variant, however, particularly with regard to its apparently low level of expression. Other splice variants include GR-P (alternatively known as GR delta), which lacks exons eight and nine (2), and a group of exon 2 splice variants (3). We are currently studying an evolutionarily conserved splice variant, GR gamma, that utilises the first three bases of the intron separating exons three and four to code for an additional arginine that is located in the DNA binding domain of the receptor (4).

An additional level of complexity in GR variants is introduced by the reported use of alternative initiating codons (5) which leads to isoforms with different amino-termini, each of which may regulate both a common and a unique set of genes.

Although a unique function has not yet been definitively assigned to any of these variants, the important possibility arises that some or all of them could fulfill distinct functions, perhaps providing a route to pharmacological modulation of signalling through different pathways.

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SA4

Glucocorticoid receptor and chromatin remodelling

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In the eukaryotic nucleus, genetic information is stored within the intimate association of DNA and histone proteins, resulting in a dynamic polymer called chromatin. The fundamental structural unit of chromatin is the nucleosome which consists of ~146 bp of DNA wrapped around an octamer of histones containing two copies each of four core histones, H2A, H2B, H3 and H4. We have developed model systems to study the mechanisms by which steroid receptors control many physiological activities by regulating gene expression within this chromatin organization. Our studies have focused on the glucocorticoid receptor (GR) and its ability to remodel chromatin which is mediated by BRG1 ATPase as part of the human SWI/SNF complex. We demonstrate that transactivation from a chromatin template requires an intact BRG1 remodelling activity, and this activity cannot be substituted by other ATP-dependent remodelling proteins. Further, we have designed, characterized and utilized BRG1 truncation and deletion mutants in a series of assays which allow independent evaluation of transcriptional activation and chromatin remodelling by the GR. Our results suggest BRG1 may contain previously uncharacterized functional motifs important for transcriptional activity. Finally, they reveal that protein-protein interactions both within the complex as well as between the remodelling complex and nuclear receptors necessary and sufficient for glucocorticoid receptor functions *in vivo*.

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SA5

Transcription factor mobility and promoter progression

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The classical view of nuclear receptor action postulates the static binding of liganded receptors to the promoter. We have discovered, however, that nuclear receptors interact dynamically with regulatory elements in living cells, and have proposed the hit-and-run hypothesis for receptor function. Two separate, ATP-dependent mechanisms have been implicated in rapid exchange, the first related to chromatin remodelling, and a second involving molecular chaperone action.

We have also observed that steroid receptor responsive promoters move through a complex series of activity states, a phenomenon we term promoter progression. Genome-wide profiling of glucocorticoid receptor (GR) regulated loci reveals several classes of response, including genes that are transiently activated and genes that are transiently repressed.

Thus receptor action either leads to a series of events programmed into each promoter, or the receptor and/or associated factors are subject to a time-dependent modification of their activity states. These findings illustrate the complexity of the GR response and indicate the dynamic nature of hormone action throughout the eucaryotic genome.

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SA6

Hypothalamic integration of neuroendocrine and autonomic responses to stress

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The hypothalamic paraventricular nucleus (PVN) is the major brain site that synthesizes hypophysiotropic corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) to initiate the neuroendocrine stress response to various external or internal challenges. Additional cell clusters in the PVN give rise to long descending projections to the brain stem and spinal cord. These neurons are in the position to govern stress-related autonomic responses. Retrograde tracing studies using neurotropic viruses identified these neurons as elements of neurocircuits regulating sympathetic outflow to various organs affected by stress. Activation marker c-fos revealed challenge-specific activation of neurosecretory and autonomic-related neurons in the PVH. Much is known about the location, chemical signatures and secretory capabilities of hypothalamic effector neurons that govern the neuroendocrine stress response; however, how these come to be situationally linked to autonomic command neurons to form operational circuits during challenge, is poorly understood. There is a bidirectional communication between neurocircuits regulating feeding and stress. On one hand, stress has a significant impact on food intake and metabolism, while energy status and availability of fuels affect the sensitivity to stressors, expression of hypothalamic stress-related neuropeptides as well as determining coping strategies. As a recent example from our laboratory, chronic exposures to sucrose of adrenalectomized (ADX) or control rats resulted in activation of AVP gene expression in the autonomic-related projection neurons of the PVH. However, this challenge did not affect CRH and AVP transcription induced by the withdrawal of the steroid negative feedback signal in the neurosecretory neurons of the medial dorsal parvocellular subdivision. AVP hnRNA induction during chronic sucrose loading was confined to the ventral medial parvocellular subdivision; however, cells in the dorsal parvocellular part of the PVN that project to the spinal cord were not responsive to metabolic challenge. Autonomic-related neurons in the PVN are involved in the regulation of different fat depots. White (WAT) and brown (BAT) adipose tissue represent functionally distinct compartments of lipid storage and fuel consumption, respectively. We have applied dual viral transneuronal tracing strategy using isogenic recombinant strains of the pseudorabies virus to identify neurons in the rat hypothalamus that innervate WAT and/or BAT. In addition to various brain stem neurons, a high number of double-labelled neurons were identified in the autonomic-related subdivisions of the PVN, suggesting coordinated control of fat depots by autonomic command neurons during challenge.

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SA7

Limbic-hypothalamic neurocircuits controlling the HPA axis stress responses

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The hypothalamo-pituitary-adrenocortical (HPA) axis is a primary stress-response system in all vertebrates. The end-products of HPA activation, glucocorticoids, serve the general function of redirecting bodily resources to meet a real or perceived challenge. However, prolonged glucocorticoid secretion has deleterious effects on metabolism, immune function and behaviour, making control of HPA activity a priority for the organism. This control is exerted in large part by limbic structures in the brain. Our studies indicate that the amygdala, hippocampus and prefrontal cortex play major roles in regulating HPA axis to acute stress. The amygdala is primarily stress excitatory, whereas the hippocampus has an inhibitory influence on HPA activity. The role of the prefrontal cortex is considerably more complex; its prelimbic region is primarily stress inhibitory, whereas the infralimbic region may participate in stress activation. All of these regions exert their influence via subcortical relays to hypothalamic paraventricular nucleus (PVN) neurons controlling the HPA response, allowing convergence of information from multiple limbic sources prior to the PVN. In contrast, chronic stress-induced enhancement of HPA axis activation is associated with neuroplastic changes at multiple levels, including limbic forebrain sites, brainstem systems and the PVN proper. It is likely that chronic stress-induced alterations in limbic-PVN circuitry play a major role in stress-related HPA axis abnormalities and are involved in the pathophysiology of numerous stress-related disease states, such as depression and PTSD.

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SA8

Transmitter systems generating an integrated stress response

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A stressful stimulus will evoke a number of coincident neuroendocrine, autonomic and behavioural responses which serve both to minimize the potentially damaging effects of the threat and to restore homeostasis. Understanding how this integrated response is generated requires the elucidation of the neural pathways which transmit the stressful stimulus to the effectors systems, and the identification of transmitters that play pivotal roles in these pathways. This paper will present a conceptual framework that integration of the stress response depends on a hierarchy of interacting response networks, and that particular transmitters have a pre-eminent role in mediating or modulating network activation. Importantly these transmitters offer potential targets for pharmacological modulation of stress physiology.

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.

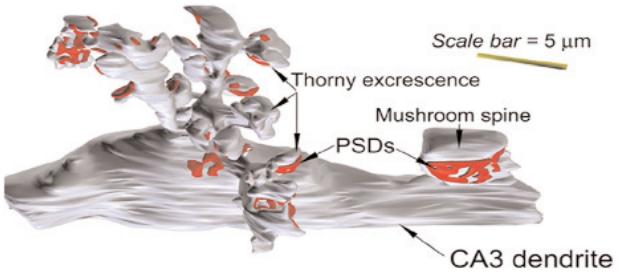

Figure 1. A 3-dimensional reconstruction from 150 serial ultrathin sections of a 20 µm segment of dendrite from CA3 with thorn excrescences. Note that the thorns show post-synaptic densities (PSD) in red, and there is also a single mushroom spine, with a PSD (scale bar, 5µm).

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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.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

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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Central mechanisms underlying attenuated responses to stress during late pregnancy

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Hypothalamo-pituitary-adrenal (HPA) axis responses to stress are compromised in disease states but under selected circumstances responses are also altered in healthy physiological states, such as during reproduction. Corticosterone secretion is attenuated in response to physical and psychological stressors in late pregnancy, parturition and lactation and may provide protection for the offspring from long term adverse effects of glucocorticoids and alter metabolic processes, balancing energy requirements of the mother and offspring. Since in the rat and mouse the responses of hypothalamic paraventricular neurones and selected inputs are also attenuated, central mechanisms underlie the HPA axis hyporesponsiveness in late pregnancy. However, enhanced glucocorticoid feedback cannot explain the adaptations in responsiveness to stress (1), as it can in disease states such as depression. We have investigated whether the attenuated responses to stress are mediated by two key central systems that regulate HPA axis responses in rats: oxytocin and monoamines. Central oxytocin inhibits the HPA axis in virgin rats, and central oxytocin release and receptor expression are enhanced in late pregnancy and parturition; however, increased oxytocin inhibition does not explain the attenuated HPA axis responses (2). In contrast, monoamines such as noradrenaline strongly drive paraventricular (PVN) neurone responses to stress. Using a combination of neuropharmacological, neuroanatomical and microdialysis studies, we have shown that noradrenergic inputs to the PVN are reduced and that PVN neurones are less sensitive to noradrenaline in late pregnancy (3), in response to swimming- or IL1β-induced stress. The opioid antagonist, naloxone partially restores HPA axis responses to stress and administered directly into the PVN also restores noradrenaline release. Thus, opioids presynaptically restrain the noradrenergic input to the HPA axis (4), contributing to the stress hyporesponsiveness. Therefore, profound adaptations occur in the brain during pregnancy to facilitate maternal accommodation of the fetus(es), and stress hyporesponsiveness, including underlying reduced noradrenergic signalling, persists into lactation (5). In summary, attenuation of the noradrenergic system underlies attenuated HPA axis responses perinatally, and is so far the only reported central transmitter mechanism that can explain hyporesponsiveness of the HPA axis at this time.

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SA17

Formyl peptide receptors in the neuroendocrine system - potential targets for pro-inflammatory and anti-inflammatory mediators

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The N-formyl peptide receptors (FPRs) are a family of G-protein coupled receptors that respond to pro-inflammatory N-formylated bacterial peptides (e.g. formyl-Met-Leu-Phe, fMLP) and, thus, contribute to the host response to bacterial infection. A growing body of evidence suggests that some members of this receptor family may also be targets for certain anti-inflammatory molecules, e.g. lipoxins and annexin 1 (ANXA1), a mediator of glucocorticoid (GC) action in the host defence system. To explore further the potential role of FPRs in mediating ANXA1 actions, we have focused on the pituitary gland, where ANXA1 has a well-defined role as a cell-cell mediator of the inhibitory effects of GCs on the secretion of corticotrophin (ACTH), and used well-established *in vitro* rodent preparations as experimental models together with molecular, pharmacological and transgenic approaches. RT-PCR analysis identified mRNAs for four FPR family members in the mouse anterior pituitary gland, Fpr-rs1, Fpr-rs2, Fpr-rs6 and Fpr-rs7. Functional studies confirmed that like GCs, ANXA1 and two ANXA1-derived peptides (ANXA11-188 and ANXA1Ac2-26) inhibit the evoked release of ACTH from rodent anterior pituitary tissue *in vitro*. The actions of ANXA1 were mimicked by lipoxin A4 (LXA4, 0.02-2μM, a lipid mediator with high affinity for Fpr-rs1) and by high (1-100μM) but not lower (10-100nM) concentrations of fMLP. Boc1 (100μM), a non-selective FPR antagonist, effectively antagonised inhibitory effects of dexamethasone, ANXA11-188, ANXA1Ac2-26, fMLP and LXA4 on ACTH release, although at a lower concentration (50μM) it was without effect. The suppressive effects of dexamethasone or ANXA1Ac2-26 on ACTH release were not affected by Fpr1 gene deletion. Similarly, at low concentrations sufficient for selective activation of Fpr1 (10-100nM), fMLP failed to modify ACTH release. Together the results suggest that the actions of ANXA1 in the pituitary gland are independent of Fpr1 but may involve other FPR family members, in particular, Fpr-rs1. They thus provide the first evidence for a role of this receptor family in the regulation of neuroendocrine function.

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SA18

Glucocorticoids and enhanced memory for emotionally arousing experiences

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Extensive evidence indicates that glucocorticoid hormones administered or released during or shortly after training on emotionally arousing tasks enhance the long-term storage of this newly acquired information. Findings of our laboratory indicate that the basolateral complex of the amygdala (BLA) is a critical component of the neural circuitry mediating emotional arousal and stress hormone effects on memory consolidation [1]. Evidence that lesions of the BLA block the modulatory effects of systemic or intrahippocampal glucocorticoid administration on memory consolidation suggests that BLA activity is essential for enabling glucocorticoid effects in other brain regions to modulate memory consolidation. Furthermore, the finding that a beta-adrenoceptor antagonist infused into the BLA blocks memory enhancement induced by posttraining systemic or intra-cerebral glucocorticoid administration indicates that noradrenergic activation of the BLA is a co-requirement in regulating glucocorticoid-induced modulatory influences on memory consolidation. These findings are consistent with recent evidence suggesting that glucocorticoids do not uniformly modulate memory of all kinds of information but, rather, preferentially influence the consolidation of emotionally arousing information. In recent experiments investigating glucocorticoid effects on memory of object recognition training we found that glucocorticoid effects on memory consolidation depend on emotional arousal because of such critical interactions with training-induced noradrenergic activation of the amygdala [2]. However, glucocorticoids not only influence the formation of long-lasting memory; there is now extensive evidence that they impair the retrieval of previously acquired information [3]. In recent experiments we found that the integrity of the BLA and noradrenergic neurotransmission within the BLA are also essential for enabling such glucocorticoid effects on memory retrieval impairment [4]. These findings are in accordance with recent evidence suggesting that glucocorticoids also require a certain degree of emotional arousal in influencing memory retrieval. Stress exposure or glucocorticoid administration further impairs working memory, a dynamic process whereby information is continuously updated. Working memory relies on the medial prefrontal cortex (mPFC). We recently reported that the BLA interacts with the mPFC in regulating glucocorticoid effects on delayed alternation performance in a T-maze, a task commonly used to investigate spatial working memory functions in rodents. A glucocorticoid receptor agonist infused into the mPFC induces impairment in working memory, and BLA lesions block the working memory impairment induced by the glucocorticoid receptor agonist [5]. Additionally, systemic administration of the beta-adrenoceptor antagonist propranolol blocks glucocorticoid-induced impairment of working memory. As our findings indicate that BLA influences on other brain regions in regulating glucocorticoid effects on memory are not restricted to consolidation of long-term memories but extend to memory retrieval and working memory, they provide compelling evidence that the BLA is part of an integrated network of cortical and subcortical brain regions engaged in regulating different, and often opposite, stress hormone effects on memory processes.

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