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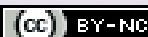
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Neural regulation of the stress response: glucocorticoid feedback mechanisms

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Abstract

The mammalian stress response is an integrated physiological and psychological reaction to real or perceived adversity. Glucocorticoids are an important component of this response, acting to redistribute energy resources to both optimize survival in the face of challenge and to restore homeostasis after the immediate challenge has subsided. Release of glucocorticoids is mediated by the hypothalamo-pituitary-adrenal (HPA) axis, driven by a neural signal originating in the paraventricular nucleus (PVN). Stress levels of glucocorticoids bind to glucocorticoid receptors in multiple body compartments, including the brain, and consequently have wide-reaching actions. For this reason, glucocorticoids serve a vital function in negative feedback inhibition of their own secretion. Negative feedback inhibition is mediated by a diverse collection of mechanisms, including fast, non-genomic feedback at the level of the PVN, stress-shut-off at the level of the limbic system, and attenuation of ascending excitatory input through destabilization of mRNAs encoding neuropeptide drivers of the HPA axis. In addition, there is evidence that glucocorticoids participate in stress activation via feed-forward mechanisms at the level of the amygdala. Feedback deficits are associated with numerous disease states, underscoring the necessity for adequate control of glucocorticoid homeostasis. Thus, rather than having a single, defined feedback 'switch', control of the stress response requires a wide-reaching feedback 'network' that coordinates HPA activity to suit the overall needs of multiple body systems.

Key words: Hypothalamo-pituitary-adrenocortical axis; Corticotropin-releasing hormone; Glucocorticoid receptor; Amygdala; Hippocampus; Prefrontal cortex

Endocrine stress responses: hypothalamo-pituitary-adrenocortical axis

Appropriate responses to stress promote survival by altering physiological processes and behavior. The principal endocrine component of the stress response involves activation of the hypothalamo-pituitary-adrenocortical (HPA) axis, which involves a neuroendocrine cascade culminating in the synthesis and secretion of glucocorticoids (primarily cortisol in humans and primarily corticosterone in rats, mice and other species). The primary physiological action of glucocorticoid signaling is redistribution of energy, increasing availability of fuels in order to promote survival capacity in the face of real or perceived threats (i.e., stressors). The largely catabolic actions of glucocorticoids require tight control, lest the organism experience long-term changes in metabolic function that can have deleterious actions. Regulation of glucocorticoid secretion is therefore subject to feedback control by, as we shall see below, a diverse

and situationally regulated array of glucocorticoid signaling mechanisms.

Activation of the HPA axis is controlled by a relatively small set of parvocellular neurons located in the paraventricular nucleus of the hypothalamus (PVN). Upon adequate stimulation by stress or circadian drive, these neurons release neural factors, such as corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), into the hypophyseal portal circulation. These factors then travel to the anterior pituitary and cause release of adrenocorticotrophic hormone (ACTH), which is released into the systemic circulation and causes synthesis and secretion of glucocorticoids by the adrenal cortex. Once released, glucocorticoids are able to bind high-affinity mineralocorticoid receptors (MR) or lower-affinity glucocorticoid receptors (GRs), which function as ligand-gated transcription factors to positively or negatively regulate gene expression (believed to be a primary glucocorticoid effect on homeostasis) (1). The affinity of the MR causes them to be extensively bound at low

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circulating levels of glucocorticoids, and they are thought to be important in ambient glucocorticoid signaling processes (e.g., controlling basal secretion across the circadian cycle) (2). In contrast, the GR is bound only during periods of high circulating glucocorticoid levels, as experienced during stress. Given this binding profile, it is not surprising to learn that the majority of glucocorticoid negative feedback mechanisms are mediated by GRs (1).

The ability for glucocorticoids to close the feedback loop via the GRs makes for a logical and compelling mechanism for control of stress responses. However, as we shall see, glucocorticoid signaling has several nuances that lend both redundancy and complexity to the HPA regulatory process.

Glucocorticoid 'fast' feedback: non-genomic mechanisms

Given the traditional view that glucocorticoids work by changing gene transcription, it is always surprising to learn of the relative speed by which negative feedback occurs. From landmark work beginning in the 1960s, we know that the most powerful glucocorticoid inhibition of the HPA axis occurs within minutes (3,4). This time-frame is far too fast to be mediated by genomic effects and occurs at the level of the CNS, an assumption proven by Keller-Wood and Dallman (5). Thus, rapid feedback must be mediated by non-genomic actions, perhaps working at or near the cell membrane.

The mechanism underlying fast feedback remained enigmatic until only recently. Elegant electrophysiologic studies indicate that glucocorticoids bind membrane receptors on PVN CRH neurons, eliciting an intracellular cascade that mobilizes the synthesis of endocannabinoids (Figure 1). Endocannabinoid release then causes presynaptic inhibition of glutamate release, which reduces the neural activity of parvocellular neurons (6). Our group has since evaluated this mechanism *in vivo*, and documented that local injection of a cannabinoid receptor 1 antagonist can block negative feedback inhibition of ACTH and corticosterone release (7). Local infusion of a membrane-impermeant glucocorticoid conjugate (dexamethasone bound to bovine serum albumin (BSA)) is equally effective as an unconjugated steroid in inhibiting HPA axis stress responses, suggesting that fast feedback effects are mediated by glucocorticoid binding at or near the membrane (7).

The receptor mediating fast feedback has yet to be identified. Research by Moore's group (8) suggests the existence of a G-protein-coupled 'membrane GR' (mGR) that is responsible for the fast effects of glucocorticoids on behavior (e.g., the clasping reflex in the newt) (8). A membrane-bound receptor was also proposed by Di et al. (6), based on evidence that glucocorticoid fast feedback was not blocked by MR or GR antagonists. However, the GR antagonist used in their study (mifepristone) causes

nuclear translocation of the GR and may be specific for genomic mechanisms of receptor signaling (9), leaving a 'membrane' action of the traditional GR open to possibility. Indeed, recent research suggests that mice bearing GR deletion in the PVN (targeted by an Sim1 promoter-cre recombinase driver crossed with mice bearing GR^{flox} alleles) do not exhibit rapid inhibition of PVN neuronal excitation *in vitro* (10), suggesting the possibility that fast feedback is indeed mediated by the classical GR. In support of this hypothesis, electron microscopy studies have documented localization of GR immunoreactivity to the cell membrane (11,12). Nonetheless, at present, the true identity of the membrane receptor remains to be determined.

Neural inhibition of stress responses: role of limbic structures

A rich literature ascribes feedback control of the HPA axis stress responses to limbic system structures, including the hippocampus and the medial prefrontal cortex (Figure 1) (see Refs. 13-15). Electrical stimulation of the hippocampus is sufficient to reduce circulating glucocorticoid levels, consistent with an inhibitory effect of hippocampal activation on the HPA axis (14). Inhibition of the stress responses of the HPA axis is also observed following chemical stimulation of the prelimbic division of the prefrontal cortex (16). Lesion studies indicate that destruction of the hippocampus (particularly its primary ventral output, the ventral subiculum) prolongs HPA axis responses to acute stressors of a psychogenic nature (i.e., stressors that signal potential, rather than emergent threats to homeostasis) (17). More recently, similar findings have been reported for the medial prefrontal cortex, where lesions delay shut-off of HPA axis responses to psychogenic stress (18-20). In both cases, the inhibitory mechanism requires an intermediary synapse to translate glutamatergic output into GABAergic inhibition at the PVN (14,15). The timing of response prolongation following either prefrontal or hippocampal damage is 1-2 h post-stress, suggesting a probable genomic mechanism.

Both the hippocampus and prefrontal cortex richly express GR (21), suggesting that inhibition of stress responses by these regions is mediated by glucocorticoid feedback. In the case of the prefrontal cortex, local implants of corticosterone are sufficient to reduce HPA axis responses to psychogenic stress, but not to stressors that signal a true physiologic threat (e.g., respiratory challenge caused by ether inhalation) (18). Evidence for local glucocorticoid regulation of hippocampal inhibition is less well established (possibly due to the difficulty in locally administering glucocorticoids across a large and elongated structure).

The role of the GR in limbic feedback regulation of the HPA axis was addressed in a series of studies using mice bearing deletion of the GR in the limbic forebrain (FBGRKO

mice) (using a CamKII alpha promoter-cre recombinase driver mouse crossed with mice bearing GRfloxed alleles). The FBGRKO mouse has GR deleted in the cerebral cortex, hippocampus and basolateral amygdala, but retains GR expression in regions such as the central and medial amygdaloid nuclei and, importantly, the PVN (as well as in all peripheral tissues) (22,23). Stress testing reveals that FBGRKO mice have delayed shut-off of HPA axis responses to psychogenic, but not systemic stressors, and are deficient in dexamethasone suppression of the circadian corticosterone peak (22,23). While these mice

cannot distinguish between actions at the prefrontal cortex vs the hippocampus, the data provide evidence for limbic forebrain GR control of negative feedback inhibition of the HPA axis. Importantly, the data also indicate that forebrain GR feedback is stressor-specific: glucocorticoid inhibition of the HPA axis is only seen when the stressful stimulus actively engages the brain structure(s) in question.

The relative contribution of the hippocampus vs prefrontal cortex was recently addressed by Radley and Sawchenko (24). Reasoning that the hippocampus and prefrontal cortex are interconnected, these investigators

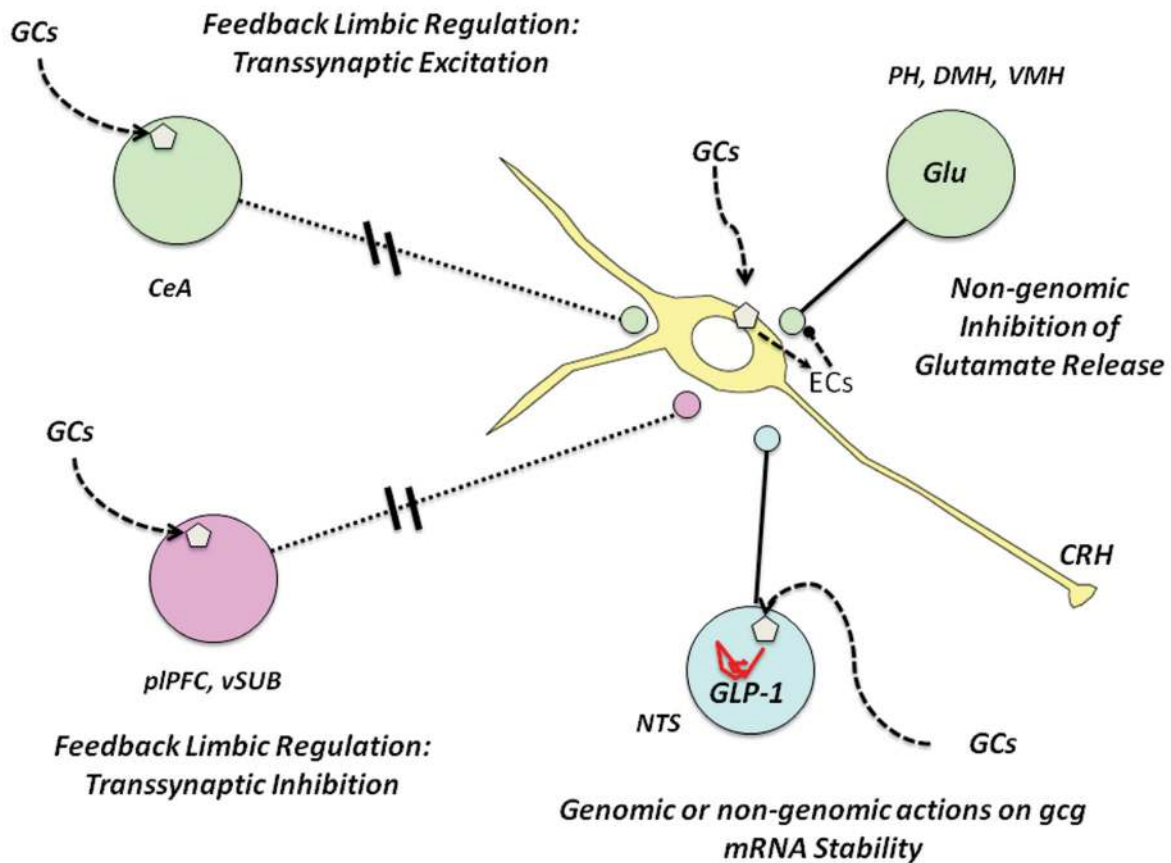


Figure 1. Glucocorticoid (GC) signaling mechanisms regulating paraventricular nucleus (PVN) corticotropin-releasing hormone (CRH) neurons. Glucocorticoid negative feedback can generally be divided into three interacting domains. GCs provide rapid, nongenomic inhibition at the PVN, mediated by endocannabinoid (EC) inhibition of PVN glutamate inputs from regions such as the posterior hypothalamus (PH), dorsomedial hypothalamus (DMH) and ventromedial hypothalamus (VMH; upper right). Forebrain genomic GC signaling is also a key component of feedback regulation, mediated via the prelimbic division of the prefrontal cortex (pIPFC) and ventral subiculum (vSUB). These structures have little or no direct interactions with the PVN and require intermediary synapses in PVN-projecting cell groups (broken lines). Glucocorticoids act on limbic output circuits (indicated in red) to inhibit the PVN (lower left). Glucocorticoids appear to act by yet a third mechanism to destabilize mRNAs (preproglucagon, *gcg*) encoding HPA-activating neuropeptides such as glucagon-like peptide-1 (GLP-1), thereby removing excitatory input to the PVN (lower right). Finally, GCs may also play a role in stress excitation, mediated by transsynaptic inputs from regions such as the central amygdaloid nucleus (CeA; upper left). NTS = nucleus tractus solitarius.

used lesion and anatomical studies to determine whether these structures controlled HPA axis function in series or in parallel. The data indicate that the prefrontal cortex and hippocampus innervate common subcortical targets, and that combined lesions had additive effects on HPA axis hyperactivity, suggesting that both regions are involved in the regulatory process, likely acting in parallel.

Ascending feedback mechanisms: inhibition by subtraction?

Feedback control of stressors signaling emergent homeostatic challenges is not well understood. In fact, Keller-Wood and Dallman (5) suggest that some stimuli, such as ether exposure, may not be subject to direct feedback, perhaps due to the necessity of glucocorticoids for meeting systemic challenges. Drive of the HPA axis by homeostatic imbalance takes several forms: sensing peripheral chemical imbalances by circumventricular organs (e.g., elevated angiotensin II in the subfornical organ, elevated circulating cytokines in the area postrema); sensing changes in metabolism via altered leptin/insulin signaling in the mediobasal hypothalamus, and relaying neural signals from the lungs, heart, gut and other internal organs via visceral sensory afferents (14). The latter mechanism prominently involves noradrenergic as well as non-catecholaminergic relay neurons in the nucleus tractus solitarii (NTS), which sends direct excitatory projections to CRH neurons of the PVN (15). Importantly, the NTS receives information from limbic regions as well as the periphery, and may be a site of general stress response coordination (15).

Neurons of the NTS express GR, and are thus plausible targets for glucocorticoid modulation of HPA axis responses (25). Currently, data directly exploring NTS glucocorticoid feedback and HPA axis function are lacking. However, recent research from our group demonstrates that expression of the preproglucagon (*gcg*) gene, which encodes the HPA-excitatory neuropeptide glucagon-like peptide 1, is rapidly down-regulated by stress-induced glucocorticoid secretion (26). Down-regulation of *gcg* expression occurs within 30 min of stress or glucocorticoid exposure, and is independent of effects on gene transcription. These data suggest that effects of stress and glucocorticoids are non-genomic, probably mediated by *gcg* mRNA destabilization and degradation. Importantly, loss of *gcg* gene expression in NTS is accompanied by a stress-induced depletion of the GLP-1 peptide in the PVN, suggesting reduced capacity for PVN excitation by a subsequent stressor (26). Loss of a potential excitatory peptidergic species is reminiscent of the stress-refractory period seen in the hours following acute stress, wherein subsequent stimulation cannot induce an HPA axis stress responses (see Ref. 5). While ascribing a role for the brainstem in stress refractivity is speculative, the data suggest that glucocorticoids modulate the capacity of excitatory input to the PVN via a non-genomic mechanism

targeting RNA stability, a mechanism distinct from either genomic modulation or membrane signaling.

The other side of feedback: feed-forward mechanisms?

To this point, we have focused largely on inhibitory effects of glucocorticoids on HPA axis activity. However, it is clear that there is another side to glucocorticoid signaling in the brain, wherein stress-excitatory regions, such as the amygdala, may be activated by the hormone. For example, stress exposure promotes CRH release in the central amygdala (27), which is linked to enhanced fear and anxiety. Stress-induced release of CRH is blocked by the GR antagonist mifepristone, indicating a role for the GR in amygdalar CRH secretion in response to stressors (28). Notably, the central amygdaloid nucleus is linked to excitation of the HPA axis, particularly following stimuli signaling homeostatic disruption (29). Region-specific knockdown of the GR in the central amygdaloid region impairs fear conditioning and reduces HPA axis responses during cued recall testing, suggesting that glucocorticoid signaling is vital for the integration of fear memories and related HPA drive (30). While the role for the amygdaloid GR in acute feed forward regulation of the HPA axis remains to be established, these data suggest that glucocorticoids may be involved in generation as well as inhibition of glucocorticoid release.

Chronic glucocorticoid exposure and chronic stress increase expression of CRH mRNA in the central amygdala (31,32), suggesting that these neurons may be 'recruited' during chronic stress and promote development of chronic stress-related pathologies. Notably, the central amygdaloid nucleus is among a short list of brain regions that show enhanced activation by an acute stress after chronic stress exposure, suggesting a role in stress sensitization. Together, these data suggest that feed-forward actions of glucocorticoids may be involved in the generation of pathological responses in the context of chronic stress.

Dynamic regulation of feedback mechanisms

Negative feedback regulation of the HPA axis is subject to regulation via any number of mechanisms, most of which affect GR function. Animal studies link deficits in negative feedback to reduced glucocorticoid signaling in limbic regions such as the hippocampus. For example, chronic stress paradigms that generate HPA axis hyperactivity and deficits in dexamethasone suppressing are associated with reduced GR expression in the hippocampus and prefrontal cortex (33-35). Early life manipulations (maternal deprivation) cause lower GR expression in hippocampus and cortex, resulting in prolonged responses to stress and deficient glucocorticoid feedback (36). Finally, forebrain deletion of the GR (see above) inhibits dexamethasone suppression of the HPA axis (22).

There are also conditions that enhance feedback efficacy. Early life handling and/or attentive maternal care increases GR expression in rodents and reduces HPA axis stress responses later in life, phenomena linked to epigenetic modification of the GR promoter (37). In addition, recovery from chronic variable stress exposure generates a period of reduced HPA axis reactivity to acute stress (38), suggesting a compensatory enhancement of feedback regulation of the HPA axis. However, behavioral studies note impaired extinction and exaggerated reinstatement of conditioned fear during 'recovery' from chronic variable stress (39), effects suggesting long-term pathological changes in stress reactivity. The relationship of behavioral deficits to attenuated corticosterone responses remains to be determined.

Feedback and pathology

Glucocorticoid dyshomeostasis is heavily represented in disease states, including stress-related disorders such as depression and post-traumatic stress disorder (PTSD). The nature of glucocorticoid deficits varies among diseases; for example, in the case of (melancholic) depression, glucocorticoid levels tend to be elevated, whereas PTSD is linked to pathologically low glucocorticoid levels (see Refs. 40,41).

Glucocorticoid deficits in both depression and PTSD are linked to altered negative feedback efficacy. In the case of depression, a sizable subpopulation of patients exhibit deficient glucocorticoid feedback inhibition of the HPA axis, manifest as 'escape' from dexamethasone suppression of the circadian cortisol rise (e.g., Refs. 42,43). Deficits in feedback resemble those seen in animal models of chronic stress, maternal deprivation or limbic GR knock-down (34,36,37). Of note, all of the aforementioned manipulations increase depression-like behavior (34,36,37). In humans, reduced size of the anterior cingulate cortex (a division of the human prefrontal complex) correlates with impaired dexamethasone suppression, suggesting that a link between limbic cortex and HPA axis feedback efficacy

also exists in man (44).

Reduced cortisol levels seen in PTSD patients are associated with the opposite phenomenon, i.e., enhanced glucocorticoid negative feedback (45). Patients with PTSD generally exhibit enhanced dexamethasone suppression of the HPA axis and lower basal cortisol levels. Recent data suggest that low cortisol levels may predispose individuals to the development of PTSD. Moreover, maternal PTSD is linked to lower cortisol levels in the offspring, perhaps placing individuals at a transgenerational 'risk' for developing the disorder (see Ref. 45). Of note, CSF CRH is elevated in PTSD, raising the possibility that enhanced feed-forward mechanisms may also be engaged in the disorder (46).

Conclusions

Glucocorticoid feedback regulation is a dynamic process that takes full advantage of the complexities of glucocorticoid signaling to provide a diverse and multi-faceted means of controlling stress reactivity. Inhibition of the HPA axis uses glucocorticoid signaling to 1) provide rapid shut-off of initiated responses at the cell membrane; 2) control response duration at the level of the limbic forebrain, and 3) provide long-latency attenuation of excitatory input by modulation of RNA stability. In addition to its importance in controlling day-to-day glucocorticoid exposure, feedback appears to play an important role in brain homeostasis, as deficits in glucocorticoid control have deleterious effects on mood and cognition in animal models and are cardinal endophenotypes in human diseases such as depression and PTSD. Understanding the nature of feedback control and feedback pathologies will be key to unlocking mechanisms of, and developing therapies for, stress-related disease states.

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