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Review

Temporally Tuned Corticosteroid Feedback Regulation of the Stress Axis

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Activity of the hypothalamic–pituitary–adrenal (HPA) axis is tuned by corticosteroid feedback. Corticosteroids regulate cellular function via genomic and nongenomic mechanisms, which operate over diverse time scales. This review summarizes recent advances in our understanding of how corticosteroid feedback regulates hypothalamic stress neuron function and output through synaptic plasticity, changes in intrinsic excitability, and modulation of neuropeptide production. The temporal kinetics of corticosteroid actions in the brain versus the pituitary have important implications for how organisms respond to stress. Furthermore, we will discuss, some of the technical limitations and missing links in the field, and the potential implications these may have on our interpretations of corticosteroid negative feedback experiments.

Highlights

Corticosteroids exert diverse actions across multiple timescales to regulate hypothalamic corticotropin-releasing hormone (CRH) neuron function.

Corticosteroid feedback in the hypothalamus is initiated by nongenomic actions that inhibit action-potential-independent glutamatergic synaptic transmission onto CRH neurons.

Transcription-dependent slow corticosteroid feedback has multiple outcomes to synergistically inhibit CRH neural activity and peptide release.

The temporal lag in corticosteroid access to the brain following stress challenges the notion of 'fast' corticosteroid feedback in hypothalamic neurons.

Corticosteroids exert rapid inhibitory actions at the pituitary gland and this likely represents the fastest site of negative feedback in the HPA axis.

Controlling Stress

Animals, including humans, exist in environments where there are many potential threats. Because of this, organisms are equipped with systems that allow them to respond and adapt to stressors such that they can maximize their chances of survival with minimal disruption to wellbeing. The hypothalamic–pituitary–adrenal (HPA) axis is one such system that is activated in response to real or perceived threats. The effector arm of the HPA axis is mediated by corticosteroids, which are released from the adrenal gland and act both centrally and peripherally to regulate cellular function. Within the brain, corticosteroids exert pleotropic effects on synaptic function, cellular excitability, learning and memory to name a few. However, in order to prevent excessive and prolonged corticosteroid secretion, which can be detrimental to health, corticosteroids also feedback at multiple points of the HPA axis to modulate output.

The study of corticosteroid actions in the brain has a long history [1]. However, our understanding of how corticosteroids regulate neural stress circuit excitability has been limited until recently. This review focuses on recent advances in our understanding of how corticosteroids feedback to act on hypothalamic neurons to regulate stress axis output. Furthermore, we discuss the importance of feedback on anterior pituitary corticotrophs for achieving fast inhibition and argue that the relative timing of corticosteroid feedback in the hypothalamus versus the pituitary has important implications for understanding stress-induced adaptations.

HPA Axis Overview

The neuroendocrine stress axis is primarily controlled by a population of hypothalamic corticotropin-releasing hormone (CRH) neurons located in the paraventricular nucleus (PVN) of the hypothalamus. In mammals, CRH neurons project to the external zone of the median eminence where their nerve terminals come into close contact with blood vessels. Following exposure to a threat, there is an increase in excitatory input to CRH neurons and a reduction in inhibitory synaptic tone [2]. This results in an increase in spiking activity and the subsequent release of CRH from median eminence nerve terminals into the portal blood (Figure 1). A proportion of CRH neurons also produce vasopressin [3,4], which is also secreted into the pituitary portal circulation following stress [5,6]. CRH and vasopressin act synergistically to stimulate adrenocorticotropic hormone (ACTH) release from the pituitary [7,8]. Once released, ACTH travels through the general circulation to act on cells within the zona fasciculata in the adrenal cortex to stimulate the synthesis of adrenal corticosteroids. In primates and ruminants, the predominant corticosteroid is cortisol; whereas in rodents, it is corticosterone. Much of the recent research has been conducted in rodents and therefore, we will frequently refer to the actions of corticosterone for the majority of this review. In addition to this on-demand corticosterone secretion, there is also an ongoing and pulsatile rhythm of corticosterone secretions throughout the day. The mechanisms and importance of these basal corticosteroid rhythms have been reviewed elsewhere [9].

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Corticosteroids regulate a diverse array of functions throughout the body including the regulation of growth and development, fuel metabolism, immune function, and vascular tone. Importantly, corticosteroids also freely diffuse into the brain to regulate the excitability of neurons over both short and long time scales.

Corticosteroid Signaling

Corticosterone binds to and activates both mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). Because MRs have a higher affinity, these receptors tend to remain occupied even at low, basal levels of corticosterone. In contrast, GRs have a lower affinity for corticosterone; therefore, levels of GR activation tend to dynamically track the changing levels of free corticosterone [10]. For this reason, much of the research on corticosteroid feedback mechanisms has focused on GR pathways. The expression pattern of MRs and GRs differ substantially throughout the brain. MRs are expressed in limbic brain circuits and as well as in the pituitary [10–12], whereas GRs are more widely expressed throughout the brain. However, GR expression is particularly high in the hypothalamus, hippocampus and the pituitary [10–12], suggesting that this receptor plays an important role in regulating the function of cells in these regions.

GRs belong to the superfamily of ligand-inducible transcription factors. Upon binding corticosterone, these receptors homo- or heterodimerize [13] and then translocate into the nucleus where they bind to other transcription factors or DNA response elements. Thus, one outcome of receptor activation is changes in gene transcription. This genomic mechanism of action is considered to be relatively slow, acting on the time frame of tens of minutes to hours. There are however many examples of corticosteroid actions that occur on the timeframe of seconds to minutes, which is inconsistent with the classical **genomic GR signaling** (see Glossary) mechanism [14,15]. It is this fast feedback that has been hypothesized to mediate fast shut off of the neuroendocrine stress axis following exposure to a threat.

These rapid actions of corticosterone are nongenomic and are thought to be mediated by putative membrane-associated GRs [14–16]. Interestingly, knock out of the classical GR also removes the fast, nongenomic actions of corticosterone suggesting that both fast and slow (or nongenomic and classical) pathways require the same receptor [17]. It is currently unknown how the same gene/receptor mediates both forms of corticosteroid feedback. One possibility is that the receptor may translocate from the cytoplasm to the membrane and activate G proteins [18]. Another possible explanation is that the membrane-associated GR is transcribed from a separate gene, but the expression of the membrane GR is controlled by the genomic actions of the classical GR.

Effects of Corticosterone on HPA Output

Many studies have manipulated plasma corticosteroid levels in awake rats and measured the resulting impact on HPA output [19–22]. These studies report a robust suppression of stress-evoked ACTH secretion, which manifests over a time course of minutes. However, one study observed suppression of stress-induced ACTH as quickly as 30 s following intravenously delivered corticosterone [22]. Determining the mechanisms that underlie these effects of peripherally delivered corticosteroids can be difficult due to the fact that they will act on the adrenal and pituitary, as well as in the brain. Indeed, *in vitro* pituitary studies clearly demonstrate that corticosterone can suppress corticotroph electrical excitability, calcium elevations, and ACTH secretion within minutes to tens of minutes [23–27].

The rapid suppression of ACTH release noted earlier was only observed if corticosterone was elevated prior to stress; suppression of ACTH was not observed if corticosterone was administered 5 min after stress onset [22]. This suggests that once CRH and ACTH secretion has been initiated, activation of fast GR pathways is less able to shut it off. The effectiveness of corticosterone feedback may also vary with stressor intensity and modality. Thrivikraman *et al.* reported that corticosterone treatment suppressed ACTH responses to an air puff stress but not hemorrhage [20]. This stressor-specific nature of fast feedback could be explained by differences in corticosterone sensitivity between upstream neural circuits encoding different stress modalities or differences in the effectiveness of negative feedback in modulating responses to mild versus severe stressors.

Glossary

CRISPR: CRISPR stands for clustered regularly interspaced short palindromic repeats. It is a technology that can be used to specifically edit genes.
Excitatory postsynaptic potential (EPSP): depolarizing membrane potential recorded from a postsynaptic neuron in response to an excitatory synaptic input. An inhibitory synaptic input generates a hyperpolarization referred to as an inhibitory postsynaptic potential.
Genomic GR signaling: corticosteroid actions via a cytosolic glucocorticoid receptor that regulates gene transcription and exerts slower but longer-lasting actions.
Heteronuclear RNA (hnRNA): primary transcript (or heteronuclear) RNA that is present in the nucleus before being processed into mature mRNA.
Homotypic stress: refers to repeated presentation of the same stress.
Nongenomic GR signaling: corticosteroid actions via a putative membrane-associated receptor linked to intracellular signaling cascades. The effects on cell function of nongenomic signaling are faster than genomic signaling.
Parvocellular neurosecretory cells (PNCs): neurons in the PVN that project to the median eminence. PNCs include neurons that synthesize CRH, thyrotropin-releasing hormone, and somatostatin. Prior to genetically modified rat/mouse lines, PNCs were distinguished from preautonomic and magnocellular PVN neurons during electrophysiological recordings based on their electrical cell properties.
Pro-opiomelanocortin (POMC): The *POMC* gene codes for a precursor peptide that is subsequently cleaved to produce ACTH.
Quantal synaptic transmission: neurotransmitters are released in packaged vesicles called quanta. When spontaneous transmitter release is being recorded in the presence of tetrodotoxin (to block action potentials), the individual synaptic events that are recorded are referred to as quantal.

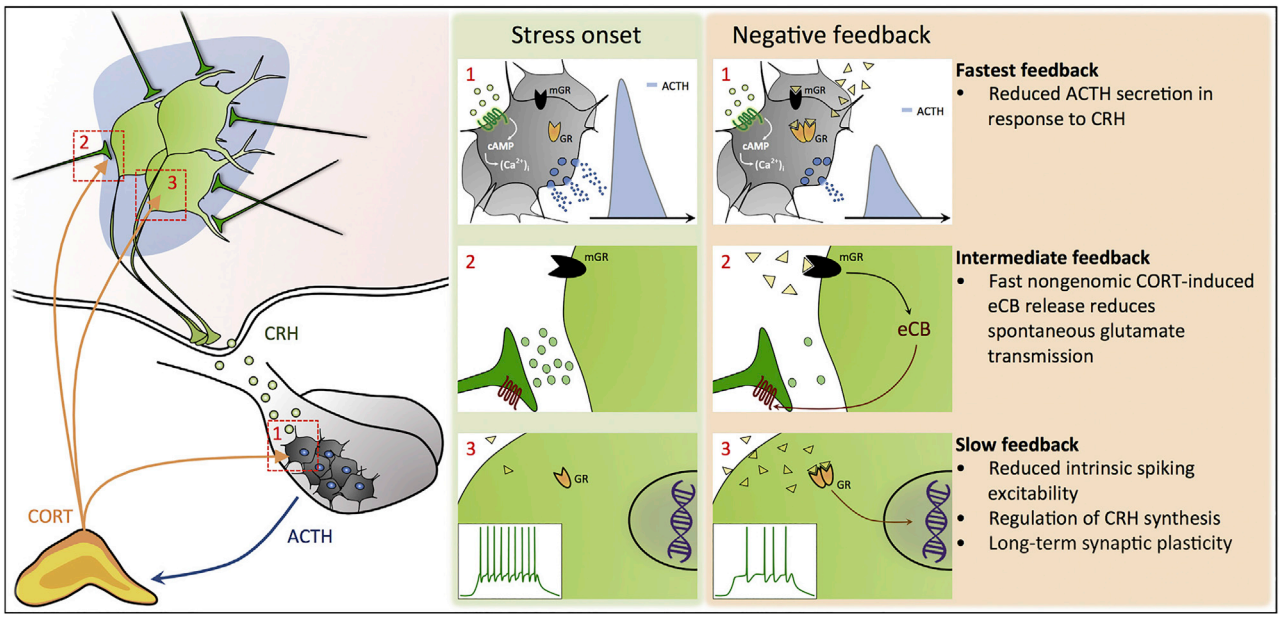

Figure 1. Timeline of Negative Feedback in the Hypothalamic–Pituitary–Adrenal (HPA) Axis.
The illustration on the left depicts corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) projecting axons down to the median eminence where the CRH peptide is released. CRH travels in the pituitary portal circulation to act on corticotroph cells in the anterior pituitary stimulating the subsequent secretion of adrenocorticotropic hormone (ACTH). ACTH travels in the blood (blue arrow) and acts on the adrenal gland to stimulate the synthesis and release of adrenal corticosteroids (orange arrow). Corticosteroids feedback onto corticotrophs in the pituitary and CRH neurons in the PVN to regulate HPA axis output. The middle and right panel illustrates events that occur at the onset of stress and following stress induced corticosteroid release. (1) The fastest site of negative feedback likely occurs at the pituitary where rapid corticosterone actions suppress ACTH secretion prior to inhibition of CRH release. (2) Following a temporal delay, corticosterone diffuses into the hypothalamus where it binds to putative membrane-associated glucocorticoid receptor (mGRs). Activation of this fast nongenomic pathway stimulates the production of endocannabinoid (eCBs) which are released from the postsynaptic neuron and suppress spontaneous glutamate transmission via presynaptic cannabinoid type 1 (CB1) receptors. (3) The genomic actions of corticosterone on CRH neurons are the slowest process. This requires activation of cytosolic glucocorticoid receptors (GRs) and changes in gene transcription, which ultimately leads to changes in intrinsic excitability, long-term synaptic plasticity, and neuropeptide synthesis. Abbreviation: CORT, corticosteroids.

To directly test the effects of glucocorticoids acting in the PVN, studies have microinjected or implanted glucocorticoids directly into the PVN of conscious rats and subsequently measured HPA axis output. These studies report suppression of stress evoked ACTH when measured between 15 min and 3 h after PVN injection [28–30]. Evanson *et al.* observed inhibition of ACTH secretion within 15 min of intra-PVN dexamethasone, but also observed similar inhibition with a nonmembrane permeable form of dexamethasone [29]. These data suggest that nongenomic GR signaling is involved [29]. Interestingly, no effects on PVN c-fos mRNA or protein immunoreactivity were observed following treatment with corticosterone or dexamethasone [29,30] (see Outstanding Questions). Local iontophoresis of corticosterone or hydrocortisone into the PVN has also been shown to inhibit the spiking activity of median eminence projecting PVN neurons recorded in anaesthetized rats [31]. However, both the magnitude and time course of the HPA axis suppression following exogenous corticosterone or dexamethasone injections need to be interpreted cautiously, as both the magnitude and kinetics of GR activation in the PVN are likely to be very different to that observed following a 'normal' stress.

An alternative approach to determine the role of PVN GR activation in shaping stress responses has been to knock out GRs from PVN neurons [32]. Many studies have achieved this by using the Sim1-cre mouse to knock out the GR from all PVN neurons. These studies have confirmed an important role for GRs within the PVN in the regulation of both basal and stress evoked ACTH/corticosterone secretion

[33–36]. However, different studies have found different magnitude effects as well as differences between the sexes [35,36]. There are several caveats with this particular knockout approach. Firstly, GR will be knocked out from birth and thus compensatory changes within the PVN will occur [35]. Secondly, the degree of knockout of the GR is not always complete [36]. Finally, Sim1 is expressed in other brain structures besides the PVN [37], and so GR will also be knocked out from these neurons. The phenotype of this knockout model also displays symptoms of Cushing's disease, which can confound the stress response data [34].

Overall, a wide variety of studies using different techniques have shown that corticosterone action on GRs within the PVN can regulate activity of the HPA axis. With the use of **CRISPR**, it is now possible to knock out receptors in defined cell populations in adult animals [38,39]. Therefore, future work that specifically knocks out GRs only from CRH neurons in the PVN of adult animals could shed additional light on the importance of this corticosterone feedback pathway in this specific neural population.

Cellular Mechanisms and Time Course of Corticosterone Negative Feedback

As corticosterone actions within the brain can regulate HPA axis output, it becomes essential to understand the cellular mechanisms of this regulation (Figure 1). While corticosterone also acts within brainstem and limbic circuits that provide input to the PVN, we will focus specifically on the cellular mechanisms shown to be important in regulating the function of PVN **parvocellular neurosecretory cells (PNCs)** and anterior pituitary corticotroph cells. Importantly, PNCs include CRH neurons as well as other PVN neurons projecting to the median eminence. The following sections will review advances in the understanding of mechanisms by which negative feedback regulates cell excitability and gene transcription.

Regulation of Synaptic Input to PNCs

Previous work has shown that corticosterone or dexamethasone, when bath applied to PNCs in brain slices, cause a reduction in the frequency of spontaneous glutamate synaptic currents [16]. This inhibition is observed within several minutes and is also observed following bath application of nonmembrane permeable GR agonists. Both these and other results (reviewed here [40]) strongly suggest the involvement of a nongenomic GR pathway. In PVN neurons, activation of this fast nongenomic pathway leads to the synthesis of endocannabinoids [41]. These signaling molecules, once synthesized, diffuse out of the cell and act on cannabinoid receptors on presynaptic nerve terminals to quickly inhibit synaptic transmission [42] (Figure 1). Indeed, corticosterone-induced endocannabinoid release has been proposed as one of the key pathways driving fast feedback suppression of CRH neuron activity following stress [43]. However, the role of spontaneous (action potential independent) glutamate transmission in controlling firing rate is unclear and it remains unknown whether corticosterone-mediated endocannabinoid release can reduce evoked, action potential dependent, glutamate release onto CRH neurons.

While corticosterone can inhibit spontaneous glutamate synaptic transmission acutely, these inhibitory effects become less robust after several days of repeated **homotypic stress** [44]. The loss of these inhibitory effects is thought to be due to a downregulation of type 1 cannabinoid (CB1) receptors. Blocking genomic GR signaling can prevent repeated stress from downregulating CB1 and hence maintain the acute inhibitory actions of endocannabinoids [44]. In this scenario, corticosterone can both inhibit excitation but excessive prolonged corticosterone exposure can impair negative feedback.

In addition to modulating glutamate synaptic transmission, corticosterone also regulates γ-aminobutyric acid (GABA) synapses. In naïve, nonstressed animals, GABA acts as an inhibitory neurotransmitter in CRH neurons, exerting a tonic inhibitory tone, which restrains CRH neuron excitability. However, the role of GABA in regulating CRH neuron function becomes difficult to predict following stress as changes in intracellular chloride levels can cause GABA to become excitatory [45,46]. This means that while suppression of inhibitory GABA transmission in nonstressed animals may elevate excitability, suppression of excitatory GABA transmission in animals that have recently experienced stress

may in fact have the opposite effect; that is lower excitability [2]. Work from Verkuyl *et al.* has shown that the frequency of **quantal** GABA synaptic currents recorded from PNCs are suppressed in brain slices collected after a restraint stress or *in vivo* injection of corticosterone [47]. This effect could also be observed when brain slices were incubated for 20 min in corticosterone and GABA synaptic events recorded 1–5 h later [47]. Removal of endogenous corticosterone with adrenalectomy had the opposite effect; resulting in an enhanced frequency of quantal GABA synaptic currents [48]. The intracellular pathways mediating these effects remain to be determined. Another laboratory has shown that incubation of brain slices in corticosterone for 1 h has no effect on quantal GABA synaptic transmission in PNCs [49,50]; however, it does enhance extrasynaptic (tonic) $GABA_A$-receptor-mediated currents [49]. The stress-derived neurosteroid, tetrahydrodeoxycorticosterone (THDOC), can also enhance extrasynaptic GABA currents in PNCs [46]. As noted above, the impact of these changes in GABA transmission on stress axis output will be ultimately be determined by the chloride reversal potential of the neuron [45,46].

While corticosterone may have direct effects on the efficacy of synaptic transmission, it can also regulate the expression of subsequent synaptic plasticity. Normally, when GABA synapses onto PNCs are repetitively activated, they undergo a process called short-term depression [50] which renders the synapse weaker for a short period of time. While this is the case for GABA synapses onto PNCs in the naïve state, after PNCs have been exposed to corticosterone, repetitive stimulation now induces a long-lasting synaptic depression known as long-term depression (LTD). This GABA LTD is expressed presynaptically and requires release of a retrograde opioid messenger that acts on presynaptic μ-opioid receptors. This switch from short to long-term depression requires genomic GR signaling and is not able to be induced until 90 min following corticosterone exposure [50].

Overall, these studies illustrate multiple different corticosterone induced changes in synaptic function that occur over a time frame of minutes to days. While the mechanism of fast corticosterone regulation of glutamatergic transmission has been well established, the mechanisms of GABAergic regulation require further study. Of note, we have only focused on studies that have specifically implicated corticosteroids in the PVN. These synaptic changes likely represent only a small subset of synaptic mechanisms which regulate PNC excitability following exposure to stress. To date, no studies have determined if corticosterone mediated effects are restricted to distinct synaptic pathways which relay particular stress information. In the future, the use of optogenetics tools will allow for this question to be addressed.

Regulation of PNC Intrinsic Excitability

The structure of a neuron and its complement of voltage-gated ion channels determines its intrinsic excitability. Similar to synaptic strength, intrinsic excitability is not fixed and can undergo plasticity [51]. Compared to the effects on synaptic transmission, less is known regarding the mechanisms and time course of corticosterone effects on PNC intrinsic excitability. Recently, it was shown that social isolation stress can regulate the intrinsic excitability of CRH neurons in a sexually dimorphic manner [52]. Specifically, stress resulted in a longer delay to first spike due to altered kinetics of a fast inactivating potassium current. The effect of stress on delay to first spike could be mimicked by treating brain slices with corticosterone for 1 h and ultimately led to a lower overall spiking excitability.

Experiments from our laboratory have also investigated the changes in CRH neuron structure and function following chronic manipulations of corticosterone levels [53]. Following 14 days of corticosterone treatment, CRH neurons were found to summate **excitatory postsynaptic potential (EPSP)** like depolarizations less effectively, and fired fewer action potentials in response to long depolarizing current steps. This suggests that following chronic corticosterone exposure, CRH neurons would require larger or a greater number of EPSPs in order to trigger a burst of action potentials. In this study, we also assessed if corticosterone treatment could lead to alterations in the dendritic architecture of CRH neurons. While 14 days of corticosterone treatment did not change gross dendritic morphology, dendritic spine density was found to be significantly reduced [53]. This finding suggests

that in addition to changing cellular excitability, corticosterone also has the potential to regulate CRH neuron structure. Thus, the net effect of corticosterone on CRH neuron spiking output will be determined by the balance of changes in presynaptic transmitter release, changes in EPSP/IPSP integration and changes in postsynaptic membrane excitability.

Regulation of CRH Peptide Production

While corticosteroids are able to dynamically regulate neural excitability, the other major factor that determines how much CRH is released is the CRH content in the nerve terminals. This is in turn controlled by CRH gene transcription and peptide production in the cell body. Following exposure to acute stress, CRH transcript levels for both **heterogeneous nuclear RNA (hnRNA)** and mRNA are increased [30,54–59]. This response would function to increase CRH peptide production in order to replace the CRH peptide secreted during stress [60]. Following stress, one mechanism shown to suppresses CRH transcription is corticosteroid feedback. Many studies have shown that peripheral administration of corticosteroids can induce a pronounced suppression of stress induced CRH hnRNA or mRNA elevations [22,58,59,61]. Osterlund *et al.* reported that intravenous corticosterone delivered 30 s before a 30-min stress could significantly suppress PVN CRH hnRNA measured immediately after stress termination. Studies which have injected corticosteroids directly into the PVN observed a similar suppression of CRH transcript measured 1 h after PVN injection [30]. Removal of endogenous corticosterone by adrenalectomy elevates CRH mRNA in the PVN and replacement with exogenous corticosterone suppresses the adrenalectomy induced rise [62,63]. Of note, adrenalectomy also increases the spiking activity of PNCs [64]. As vasopressin secretion also plays an important role in controlling the neuroendocrine stress response [65], studies have investigated the effects of manipulating corticosteroid levels on vasopressin gene expression. Similar to CRH, adrenalectomy also increases vasopressin transcript and peptide production [3,63], which can be reversed with corticosterone treatment [61,63].

It has been estimated that following changes in mRNA levels at the soma, it would take 60–90 min before newly translated peptide reached the nerve terminal [60]. Therefore, any changes in mRNA levels would not have a notable impact on peptide output at the median eminence in the short term. Thus, changes in peptide gene expression are ideally suited for slower and long term regulation of stress axis output.

Regulation of ACTH Release

In response to CRH, corticotroph cells shift from slow spontaneous single spiking to high frequency burst firing [66], which facilitates the release of ACTH. Burst firing in corticotroph cells is dependent on large conductance calcium-activated potassium channels (BK) and this firing pattern can be blocked with the BK channel blocker paxilline [66,67]. While CRH and vasopressin can independently increase intracellular calcium concentrations in corticotroph cells [27,68], vasopressin increases single spike frequency without inducing burst firing [66]. CRH and vasopressin have also been shown to depolarize corticotrophs and other cell types through the activation of nonselective cation channels [69,70].

Corticosterone feedback inhibits both corticotroph cell excitability and ACTH release. Prior exposure to corticosterone for 30 min inhibited intracellular calcium responses to CRH and/or vasopressin [27]. Incubation of corticosterone for 90 min reduced both corticotroph cell spontaneous activity and CRH-induced burst firing [26]. Interestingly, this inhibition of corticotroph cell excitability and calcium responses to CRH is not observed with shorter duration (<5 min) incubations with corticosterone [25,26]. Acute bath applications of corticosterone can however, rapidly (within 5 min) inhibit CRH-evoked ACTH release in cultured anterior pituitary cells [25]. While the mechanism remains unknown, this fast inhibition requires the classical GR protein, as it could be blocked by RU486. Furthermore, this study also showed a rapid translocation of GR from the cytoplasm to both the membrane and nucleus in response to corticosterone [25].

These studies show both rapid and delayed forms of corticosterone feedback on pituitary corticotrophs by inhibiting the release of ACTH and corticotroph cell excitability. Consistent with the

timescales of nongenomic and genomic actions, corticosterone also exerts temporally distinct inhibitory effects on ACTH release in isolated anterior pituitary glands from hypothalamic-lesioned rats [71]. Slow or delayed forms of corticosterone feedback also regulate **pro-opiomelanocortin (POMC)** expression [72]. These data clearly show that corticosterone exerts powerful actions in the pituitary in addition to hypothalamic CRH neurons.

Missing Links and Future Perspectives

As proposed by Sayers and Sayers in 1947 [73], corticosteroid negative feedback is important for inhibiting the HPA axis following stress. Given the slow genomic actions of corticosteroids, it is suggested that fast nongenomic actions primarily mediate fast shut off. Despite the rapid manner in which corticosterone can inhibit HPA axis output, it is important to note that under normal physiological conditions *in vivo*, such fast feedback is first contingent on *de novo* corticosterone synthesis and access to the brain for ligand binding. Therefore, while corticosterone rapidly inhibits quantal glutamate synaptic transmission onto CRH neurons when applied to brain slices [17], we suggest that the time course of this inhibition in the brain following stress would be markedly slower *in vivo*.

Several studies have used microdialysis to determine the time course of corticosterone elevations within the brain following acute stress. Importantly, it was found that elevations in brain corticosterone lag blood corticosterone elevations by approximately 20 min following stress [74]. Droste *et al*. subjected rats to a 15-min forced swim stress and found that intra-hippocampal corticosterone levels took 45–60 min to reach maximal levels [74,75]. Other studies using forced swim, restraint, or foot-shock stress have reported similar findings, with brain corticosterone not reaching peak levels until 60–75 min following stress onset [76–78].

While the time taken to reach peak corticosterone levels in the brain is long, levels can begin to rise from 20 min of stress onset. It is possible that these moderate concentrations of corticosterone, prior to the peak, can have fast nongenomic actions during this rising phase. This may also involve high-affinity membrane MRs, which have also been shown to induce nongenomic effects on hippocampal glutamatergic synaptic transmission [79,80]. Interestingly, MR-selective agonists can also induce fast feedback on the HPA axis *in vivo* [81,82]. These findings suggest that despite the high occupancy of cytoplasmic/nuclear MRs at basal corticosteroid levels, membrane MRs may play a role in mediating fast feedback, which warrants further investigation.

While corticosterone is indeed a powerful regulator of hypothalamic stress circuits, due to the temporal delay in reaching the brain, negative feedback actions on neurons would be an ineffective method to quickly shut off HPA axis activity following an acute stress. As past *in vivo* studies on corticosterone feedback are largely limited to hormone measurements, these studies do not exclude the possibility that fast negative feedback occurs primarily on anterior corticotroph cells, and not CRH neurons (see Outstanding Questions). Negative feedback has previously been categorized into three time domains: fast, intermediate, and delayed [83,84]. Indeed, given the delay for corticosterone access to the brain, the pituitary gland is likely to be the first site of negative feedback following a stressor *in vivo*. Numerous studies have documented fast and robust inhibitory actions of corticosterone at the pituitary *in vitro* [23–27]. Corticosterone also rapidly inhibits ACTH release in response to CRH administration *in vivo* [82,85]. Corticosterone-mediated fast inhibition of CRH release, independently of synthesis, has also been documented in the median eminence [86], which may also serve as a strategic and effective site for fast inhibition.

Together, this suggests that the fastest feedback actions of corticosterone on the HPA axis may be mediated predominantly by regulation of ACTH release from pituitary corticotroph cells, while nongenomic corticosterone actions in the brain provides intermediary feedback. The slower delayed actions of corticosterone that manifest over several hours have the potential to exert much stronger regulation of neural circuit function as these would be predicted to synergistically regulate synaptic transmission, synaptic plasticity, ion channel expression, and peptide production. We therefore argue that while corticosterone is a powerful regulator of hypothalamic stress circuits, its greatest

impacts on CRH neuron function are likely to be temporally slow. Future studies are needed to directly address the impact of corticosterone feedback (fast and slow) on CRH activity patterns *in vivo*. This could be achieved with targeted expression of calcium or voltage sensors to stably record CRH neuron activity in freely behaving animals [87,88].

Recent studies have demonstrated novel roles for hypothalamic CRH neurons in behavioral modulation, valence coding, and pheromone communication before and after a stressful experience [87,89,90]. These innovative studies have begun to challenge and extend classical roles of CRH neurons beyond that of simple endocrine effector cells. Thus, it is important to consider how corticosteroid negative feedback could regulate central CRH neural signaling beyond the HPA axis. For example, corticosteroid induced modulation of CRH neuron firing responses may impact risk assessment as well as context-appropriate behavioral responses following stress. Based on the reasoning above, corticosteroid induced modulation of these behavioral responses would manifest more slowly than inhibition of the neuroendocrine stress response, that is, ACTH release. This suggests that the speed at which corticosteroid negative feedback regulates different components of the HPA axis may play an important role in determining the sequence of adaptations organisms exhibit following stress.

Outstanding Questions

Given that corticosteroid negative feedback can independently act on the pituitary gland prior to brain access, does ACTH release provide a good proxy of CRH neural activity?

Can corticosteroid negative feedback inhibit CRH secretion, independent of CRH neural activity and synthesis?

Is nongenomic corticosteroid feedback powerful enough to regulate CRH neural activation induced by exposure to an acute threat?

After the removal of a transient stressor, how long do CRH neurons remain active and would activity still be elevated by the time corticosteroids reach the brain?

Could corticosteroid negative feedback also impact CRH-induced changes in behavior and/or pheromone release?

How important is corticosteroid feedback at extra-PVN sites in the regulation of CRH neuron excitability over short and long time frames?

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