Dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis has been implicated in a range of affective and stress-related disorders. The regulatory systems that control HPA activity are subject to modulation by environmental influences, and stressful events or circumstances can promote subsequent HPA dysregulation. The brain is a major regulator of the HPA axis, and stress-induced plasticity of the neuronal circuitry involved in HPA regulation might constitute an etiological link between stress and the development of HPA dysregulation. This review focuses on the synaptic regulation of neuroendocrine corticotropin-releasing hormone (CRH) neurons of the hypothalamic paraventricular nucleus, which are the cells through which the brain predominantly exerts its influence on the HPA axis. CRH neuronal activity is largely orchestrated by three neurotransmitters: GABA, glutamate, and norepinephrine. We discuss our current understanding of the neural circuitry through which these neurotransmitters regulate CRH cell activity, as well as the plastic changes in this circuitry induced by acute and chronic stress and the resultant changes in HPA function.

**Keywords:** corticosteroid, glutamate, GABA, norepinephrine, neural circuits, depression, synaptic plasticity, paraventricular nucleus
In addition to CRH, the CRH cells can synthesize and release vasopressin (VP). CRH and VP in the portal circulation bind to CRH and VP receptors on a subset of cells, the corticotropes, of the anterior pituitary to stimulate the secretion of adrenocorticotropin hormone (ACTH) into the systemic circulation. The ACTH then stimulates the synthesis and systemic secretion of glucocorticoids from the adrenal cortex. Hypothalamic CRH neuronal activity is largely orchestrated by three neurotransmitters: GABA, glutamate, and norepinephrine. In this review we discuss our current understanding of how these neurotransmitter systems regulate CRH neuron activity (and consequently HPA activity), and the modulation of these neurotransmitter systems by glucocorticoids and stress. One aim of this review is to provide a framework to help guide future investigations of the synaptic regulation of CRH cells that have the potential to advance our understanding of the neurological bases for HPA dysfunction. It is also an aim of this paper to inform those who study stress-induced abnormalities in HPA activity, but who may be unfamiliar with studies of synaptic transmission, in the hope of making these studies more accessible for the purpose of enhancing communication and promoting interdisciplinary investigations.

**METHODS OF IDENTIFYING CRH CELLS**

Inasmuch as CRH cells play such a central role here, before discussing the synaptic regulation of the CRH neurons, we will briefly address the challenges in identifying the CRH cells in the course of physiological experiments. The PVN can be divided into three major cell types: the magnocellular neuroendocrine cells, which project their axons into the posterior lobe of the pituitary, the parvocellular neuroendocrine cells, which send their axons to the median eminence, and the parvocellular preautonomic neurons, which project to the brain stem and spinal cord. The CRH neurons fall into the parvocellular neuroendocrine cell type. The three cell types can be distinguished with reasonable reliability on the basis of their respective somatic sizes and shapes, dendritic morphologies, and positions in the PVN. Magnocellular neuroendocrine cells tend to have larger, more rounded somata and fewer dendritic branches, often bipolar in morphology, compared to the parvocellular neuroendocrine cells, which tend to be smaller, fusiform, and with multipolar dendritic arbors. The magnocellular neuroendocrine cells are concentrated in the lateral portion of the PVN, while the parvocellular neuroendocrine cells are located mainly in the medial region of the PVN. The dorsal-most and ventral-most regions of the PVN tend to be occupied by the parvocellular preautonomic neurons, which are intermediate in somatic size between the parvocellular neuroendocrine cells and the parvocellular neuroendocrine cells, which tend to be smaller, fusiform, and with multipolar dendritic arbors. This morphological and topographical organization of the PVN can be used to identify enriched populations of the different subtypes of PVN neurons, there is fairly extensive overlap of the anatomical characteristics of the cell types within the PVN (Simmons and Swanson, 2009), such that it does not provide for a strict distillation of the three neuron subpopulations.

Distinct electrical properties have also been characterized in the three PVN cell types and have been used to assign electrical fingerprints for the reliable identification of individual cells in *in vitro* electrophysiological studies (Figure 1). Magnocellular neuroendocrine cells generate a robust A-type voltage-dependent K+ current, which causes a prominent transient outward rectification that delays action potential generation (Tasker and Dudek, 1991). Most parvocellular preautonomic neurons generate a T-type Ca2+ current, which causes a small low-threshold spike and clustering of Na+ dependent action potentials (Stein, 2001, Luther et al., 2002). Parvocellular neuroendocrine cells can be distinguished from both magnocellular neuroendocrine cells and parvocellular preautonomic cells by the absence of both the transient outward rectification and the low-threshold Ca2+ spike (Luther and Tasker, 2000, Luther et al., 2002; Figure 1).

While useful for narrowing down the field of the three cell subtypes, these anatomical and electrophysiological approaches do not allow investigators to specifically distinguish CRH neurons from the other PVN parvocellular neuroendocrine cells, such as thyrotrop releasing hormone and somatostatin cells. Electrophysiological studies using selective antibodies for CRH and for neurotransmitters or vesicular neurotransmitter transporters have been useful for characterizing the anatomical innervation of CRH neurons and its plasticity in response to stress and adrenalectomy (Flak et al., 2009). Because basal CRH peptide expression is not robust, attempts to identify CRH neurons during electrophysiological recordings with a combination of intracellular dye injection and post hoc immunostaining for CRH have been largely unsuccessful. One method that has been useful for specifically targeting CRH cells for synaptic analysis is intracellular recording combined with single-cell, reverse transcription–PCR analysis (Di et al., 2003). This technique allows the correlation of molecular expression profiles, including CRH mRNA expression, with electrophysiological properties. Transgenic mice that express green fluorescent protein under the transcriptional control of the CRH promoter have recently become available (Alon et al., 2009) and offer the greatest promise for contributing significantly to the study of the synaptic regulation of identified CRH neurons and the synaptic plasticity of CRH neurons under conditions of stress.

**GABA INPUTS**

Origins of GABA inputs to the PVN have been identified through the use of tract tracing in conjunction with immunohistochemical staining, as well as through *in vitro* electrophysiological analyses. A retrograde tracing-immunohistochemistry study revealed four discrete origins of significant GABAergic innervation of the PVN, which are: the area surrounding the supraoptic nucleus, the anterior perifornical region, the anterior hypothalamic area, and the anterior one-third of the PVN itself (Roland and Sawchenko, 1993). There is also evidence from tract tracing studies for a prominent GABAergic projection from the bed nucleus of the stria terminalis (BNST) to the PVN that relays inhibitory signaling to CRH cells from the prefrontal cortex (Radley et al., 2009) and the hippocampus (Radley and Sawchenko, 2011). Studies employing *in vivo* ibotenic acid lesions of subnuclei of the BNST have implicated specifically the posterior medial region of the bed nucleus as an inhibitory relay from limbic structures to the PVN (Choi et al., 2007, 2008). An *in vitro* brain...
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Synaptic regulation of the HPA axis

FIGURE 1 | Distinct electrophysiological fingerprints of PVN magnocellular and parvocellular neurons. (A) Magnocellular neuroendocrine cells recorded in current-clamp mode generate an A-type K⁺ current-mediated transient outward rectification (arrow) that is generated by depolarization from a hyperpolarized holding membrane potential and delays the onset of spiking. Lower traces: current injection protocol. (B) Parvocellular neuroendocrine cells fail to generate a transient outward rectification and delay to spiking (arrow) in response to a similar current injection protocol. (C) Parvocellular preautonomic cells do not display a transient outward rectification, but generate a low-threshold spike (arrow), mediated by a T-type Ca²⁺ current, and clustered action potentials in response to a similar current injection protocol. Modified from Luther and Tasker (2000).

Slice electrophysiological study identified robust local GABAergic innervation of both parvocellular and magnocellular neurons of the PVN that originates in the perinuclear area surrounding the PVN (Boudaba et al., 1996). The perinuclear PVN area has also been postulated to serve as an inhibitory relay into the PVN from upstream limbic structures, such as the hippocampus and septum (Ziegler and Herman, 2002).

The inhibitory effect of GABA on CRH cells is mediated primarily by GABAA receptors. In situ hybridization assays detected the expression of the GABAA receptor α2, β1, and β3 subunits in nearly all the CRH cells of the PVN, and expression of α1 and β2 in about half of the CRH cells (Cullinan, 2000). Interestingly, microinjection of a GABAA receptor antagonist into the PVN is sufficient to activate CRH cells and elicit a surge in glucocorticoid secretion (Cole and Sawchenko, 2002), revealing a tonic inhibitory GABAergic input in the PVN that constrains CRH neuronal activity under basal conditions. The tonic inhibition of CRH neurons preserved in organotypic hypothalamic slice cultures containing the PVN (Balí and Kovacs, 2003), which suggests that local GABA neuronal circuitry (i.e., GABA circuitry retained through the slicing procedure) plays a central role in this mechanism. The source of local GABAergic innervation of the PVN CRH neurons is predominantly extranuclear (Figure 2), including a robust input from the perin-PVN region (Boudaba et al., 1996).

STRESS PLASTICITY OF GABA INPUTS

Acute stress plasticity of GABA inputs

The GABAergic synaptic innervation of PVN neurons undergoes significant plastic changes in response to acute stress, which alters the excitability of the parvocellular neurons and activation of the HPA axis. A 30–60 min acute restraint stress is enough to cause a significant shift in the Cl⁻ gradient across the PVN parvocellular neuron membrane via downregulation of the membrane K⁺–Cl⁻ co-transporter KCC2. This shift in the Cl⁻ gradient causes a postsynaptic attenuation of the inhibitory GABAergic inputs to these cells and leads to the disinhibition of the HPA axis (Hewitt et al., 2009). It is not known whether glucocorticoids modulate KCC2 expression, although KCC2 expression has been found to be subject to steroid modulation by sex hormones in substantia nigra neurons wherein, at postnatal day 15, GABAA receptors are hyperpolarizing in females and depolarizing in males (Galanopoulou

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Acute stress also appears to induce a long-term presynaptic plasticity in the GABAergic inhibitory innervation of PVN parvocellular neurons via a transient elevation in circulating glucocorticoids. Thus, a 30 min acute restraint stress was found to cause a persistent reduction in inhibitory synaptic inputs to putative parvocellular neurons in recordings performed in brain slices in vitro up to 5 h later, and this effect was mimicked by a previous in vivo subcutaneous injection of glucocorticoids (Verkuyl et al., 2003). These changes in inhibitory inputs were not spike-dependent, suggestive of plasticity in the GABA release probability, which was corroborated with paired-pulse analysis. An acute 20-min glucocorticoid application directly to brain slices had no rapid effect (< 20 min) on GABAergic synaptic currents (Di et al., 2003), but resulted in a similar presynaptic suppression of GABA inputs to PVN parvocellular neurons 1–3 h later (Verkuyl et al., 2005), suggesting that these presynaptic glucocorticoid actions are transcriptional and occur directly in the hypothalamus.

GABAergic synaptic plasticity may also play a role in the HPA response to hypoglycemic stress via signaling through neurotransmitters like Y (NPY). There is a high density of NPY projections that terminate in the medial parvocellular PVN (Cowley et al., 1999), many of which originate in the arcuate nucleus, a hypothalamic region that provides access to the brain of circulating nutritional signals (Wang et al., 2004). Both insulin-induced hypoglycemia (Tuchelt et al., 2000) and intracerebroventricular injection of NPY (Dimitrov et al., 2007) activate the HPA axis and cause an increase in the circulating glucocorticoid level. In vitro electrophysiological studies reported that NPY caused a decrease in the probability of GABA release onto putative parvocellular PVN neurons via actions at multiple presynaptic NPY receptors (Cowley et al., 1999). The role of HPA axis activation in response to hypoglycemia may be related to the fact that glucocorticoids increase glucose bioavailability by shifting metabolic processes towards catabolism.

Chronic stress plasticity of GABA inputs

Chronic stress and sustained changes in systemic glucocorticoid levels also lead to long-term shifts in the GABAergic synaptic innervation of the PVN parvocellular neurons. Adrenalectomy, for example, which eliminates the main endogenous source of circulating glucocorticoids, leads to an increase in the number of GABAergic synapses and in the density of GABA receptors on CRH neurons, suggesting that the loss of circulating glucocorticoids induces a proliferation of afferent GABAergic synaptic inputs to CRH neurons. Thus, quantitative electron microscopic analysis of immunocytochemically labeled GABAergic synaptic profiles revealed a >50% increase in the number of GABAergic synapses on CRH cells in the PVN of adrenalectomized rats (Mlíkos and Kovacs, 2002). An in vitro electrophysiological study provided a physiological corroboration with the finding that putative parvocellular PVN neurons undergo a significant increase in inhibitory synaptic inputs in brain slices from adrenalectomized rats, and paired-pulse analysis suggested that this effect of adrenalectomy was due to an increase in the number of GABAergic synapses (Verkuyl and Joels, 2003). Glucocorticoid replacement in this study confirmed that the effect of adrenalectomy was due to the loss of endogenous glucocorticoids. Finally, binding of radiolabeled muscimol, a selective GABAA receptor agonist, was found to increase in the hypothalamus following adrenalectomy, suggesting an increase in postsynaptic expression or membrane localization of GABAA receptors following adrenalectomy (Magowska et al., 1983).

Whereas the loss of endogenous glucocorticoids by adrenalectomy was shown to increase the number of GABAergic synapses on CRH cells, chronic stress and chronically high glucocorticoid levels were reported to suppress GABAergic inhibitory synaptic inputs to PVN parvocellular neurons. In vitro electrophysiological recordings from putative parvocellular neurons in PVN brain slices taken after 3 weeks of a chronic variable stress treatment, which leads to sustained elevated glucocorticoids (Herman et al., 1995), revealed a decrease in GABAergic inhibitory synaptic inputs to parvocellular neurons without an accompanying reduction in the probability of release of GABA, suggesting a reduced number of GABAergic synapses (Joels et al., 2004; Verkuyl et al., 2004). A confocal immunofluorescence study, however, failed to detect a reduction in the density of GABAergic synaptic boutons on CRH neurons in the medial parvocellular PVN, although these measurements were taken following a shorter exposure (1 week) to the chronic variable stress treatment (Flak et al., 2009). The acute glucocorticoid-induced reduction in GABA release probability and the chronic glucocorticoid-induced decrease in GABA synapses, in conjunction with the adrenalectomy-induced increase in GABAergic synapses, suggest that there is an inverse causal relationship between glucocorticoid levels and the efficacy of GABAergic synaptic inhibition of CRH cell activity. Both acute and chronic stress, therefore, appear to downregulate GABAergic synaptic transmission in PVN parvocellular neurons, albeit in different ways. The progression from acute stress to chronic stress plasticity in the functional inhibitory synaptic regulation of parvocellular PVN neurons appears generally to involve a transition from a modulation of GABAergic synaptic strength to a structural modification of afferent GABA inputs.

While, to our knowledge, an analysis of the changes in the density of GABAergic receptors in the PVN following chronic variable stress is still lacking, molecular studies on chronic stress-induced changes in GABAergic receptor expression in the PVN have been performed, and have produced somewhat contradictory findings. An in situ hybridization study reported a decrease in the expression of the GABAA receptor β1 and β2 subunits (Cullinan and Wolfe, 2000). Another study using a single-cell RNA amplification approach reported no change in the β receptor subunit expression, but an elevated expression of the α5 subunit and decreased expression of the δ subunit in putative parvocellular PVN neurons following chronic variable stress (Verkuyl et al., 2004). However, the absence of a change in β subunit expression in the latter study may have been a consequence of the small sample size, as the
investigators themselves concede. While the regulation of α5 and β2 GABA_A receptor subunits may suggest a stress-induced change in extrasynaptic GABA_A receptor signaling, the decrease in β1 and β2 subunits expression should lead to the downregulation of functional synaptic GABA_A receptors and/or a change in synaptic GABA_A receptor efficacy. Corresponding changes in postsynaptic GABA_A receptor sensitivity have not been reported to date.

**GLUTAMATE INPUTS**

Double immunohistochemical labeling for the vesicular glutamate transporter VGlut2 and for CRH has shown that PVN CRH neurons are densely innervated by glutamatergic fibers (Wittmann et al., 2005). Electrophysiological experiments have determined that functional local circuit glutamatergic innervation of putative parvocellular neurons originates in areas adjacent to the PVN, largely overlapping sources of peri-PVN GABAergic input, but at a much lower density (Boudaba et al., 1997; Tasker et al., 1998).

There is also evidence that glutamatergic input to PVN neurons originates within the PVN itself (Figure 2). First, CRH-, oxytocin-, and VP-expressing neurons in the PVN have all been shown to co-express VGlut2 mRNA (Hrabovsky and Lipsitz, 2008). In vitro brain slice electrophysiological recordings from putative magnocellular neurons of the PVN revealed a nonphosphoinositol-evoked stimulation of local glutamate circuits within the PVN that was spike-dependent, suggesting the presence of glutamatergic neurons in the PVN that mediate intra-PVN signaling (Daffary et al., 1998). Furthermore, intra-PVN glutamatergic circuits were also revealed in an autoradiographic study in which sources of glutamatergic input to the PVN were identified using the radiolabeled retrograde marker [3H]D-aspartate, which selectively labels glutamate neurons (Csaki et al., 2000). In addition to the peri-PVN and intra-PVN sources of glutamatergic inputs to the PVN, significant glutamatergic afferents to the PVN have also been identified emanating from the ventromedial hypothalamic nucleus, the anterior hypothalamic nucleus, the lateral hypothalamic nucleus, and the posterior hypothalamic nucleus (Bohmer et al., 1997; Tasker et al., 1998). Unexpectedly, intracerebroventricular injections of selective group I and III mGluR agonists were found to elicit an increase in circulating glucocorticoids (Lang and Ajmal, 1995; Johnson et al., 2001). Unexpectedly, intracerebroventricular injections of antagonists of the group I mGluRs were also found to trigger the activation of the HPA axis (Bradbury et al., 2003). Based on previous findings of a pre- and postsynaptic modulation of magnocellular neuroendocrine cells by group I and III mGluRs (Schraer and Tasker, 1997; Tasker et al., 1998), a similar model for the regulation of CRH cell activity by group I and group III mGluRs was proposed (Johnson et al., 2001). According to this model, group I mGluRs disinhibit CRH cell activity by suppressing the release of GABA onto CRH cells, and group II mGluRs inhibit CRH cell activity and suppression of glutamatergic neuronal output through stimulation of different GABAergic neurons, while postsynaptic group I mGluRs stimulate CRH cell activity directly.

**STRESS PLASTICITY OF GLUTAMATE INPUTS**

**Acute stress plasticity of glutamate inputs**

Glutamatergic synaptic inputs to CRH cells are suppressed by rapid glucocorticoid actions that appear to be involved in the glucocorticoid-mediated fast negative feedback of the HPA axis. Thus, corticosterone and deamethasone cause a rapid (within 3–5 min) decrease in the frequency of miniature excitatory postsynaptic currents (mEPSCs) in each of the major neuroendocrine cell types of the PVN, including in CRH cells, recorded in hypothalamic slices (Dv et al., 2003). This rapid glucocorticoid effect is mediated by a non-genomic mechanism via the activation of a membrane-associated receptor. Importantly, this glucocorticoid

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effect was blocked by antagonists and mimicked and occluded by agonists of the cannabinoid type 1 receptor (CB1, R1), suggesting the involvement of the endocannabinoid system. We recently corroborated this with the finding that the rapid glucocorticoid effect is absent in CB1, R knockout mice (Nahar et al., submitted). This was supported by the finding that glucocorticoid rapidly (<10 min) triggers the synthesis of the major endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in slices of the PVN and immediate surround (Málcher-Lopes et al., 2006). Endocannabinoids signal in a retrograde fashion (i.e., from postsynaptic membrane to presynaptic membrane) and cause the inhibition of presynaptic neurotransmitter release (Freund et al., 2003). Consistent with the endocannabinoid acting as a retrograde messenger to inhibit glutamate release at glutamate synapses, the glucocorticoid effect was prevented by blockade of G protein and protein kinase activity and Ca2+ signaling specifically in the postsynaptic cell (Málcher-Lopes et al., 2006; Harris and Tasker, 2011).

Interestingly, the glucocorticoid-induced rapid suppression of glutamate release in the PVN in vitro is not reversible in brain slice electrophysiological studies for over an hour after dexmethasone administration (Di et al., 2003), which indicates that glucocorticoids trigger a form of endocannabinoid-mediated long-term depression of synaptic excitation; long-term depression is recognized as a widespread form of synaptic plasticity in the brain that reduces the efficacy of synaptic interactions (Heifets and Castillo, 2009). The physiological significance of the extended duration of the glucocorticoid-induced suppression of excitatory inputs to CRH cells remains unknown; however, we have preliminary evidence for the desensitization to the rapid glucocorticoid-induced suppression of glutamate release in brain slices from animals that had been subjected to acute 30 min restraint stress prior to sacrifice (Jiang and Tasker, unpublished observation). This effect is likely due to the long-term depression of glutamatergic synaptic inputs by a tonic activation of presynaptic CB1 receptors via glucocorticoid-induced retrograde endocannabinoid release, since a CB1 receptor-mediated inhibitory tone was observed in glutamate inputs to parvocellular neurons from acute stressed rats, but not from unstressed rats. A downregulation of rapid glucocorticoid-induced endocannabinoid-mediated suppression of synaptic excitation was also reported in putative parvocellular neuroendocrine cells of juvenile rats subjected to a repeated immobilization stress, although the mechanism proposed was a chronic stress-induced desensitization of the cannabinoid signaling system (Wamsteker et al., 2010), rather than the increased endocannabinoid inhibitory tone that we find after single exposure to an acute stress or stress level of glucocorticoid. It remains to be determined how long the glucocorticoid-induced long-term depression of synaptic excitation lasts, but it may be in place to provide the time necessary for the peptidergic stores in the somata and axon terminals to replenish after a rapid and robust secretion of CRH and VP into the portal circulation. Also, for those mechanisms that are sensitive to rates-of-change in glucocorticoid concentration (Jacobson and Sapolsky, 1993), long-term depression of the synaptic excitation of CRH neurons may reduce CRH release to allow for clearance of circulating glucocorticoids in order to ensure the sensitivity of glucocorticoid targets to the secretion of a subsequent bolus.

N-methyl-d-aspartate receptor expression in the medial parvocellular PVN has also been reported to be modulated by a single exposure to a stress stimulus. An in situ hybridization study found that acute immobilization stress caused a ~35% increase in the expression of mRNA for the GluN1 subunit of the NMDA receptor in several brain regions, including in the medial parvocellular PVN (Bartanusz et al., 1995). The increased GluN1 expression suggests the possibility that acute stress may cause an overall increase in NMDA receptor function in the medial parvocellular PVN, since functional NMDA receptors are comprised of a requisite GluN1 subunit (Cull-Candy et al., 2001). However, a brain slice electrophysiological study demonstrated depressed NMDA receptor function in individual PVN parvocellular neurons following a 30 min acute immobilization stress ((Kuzmiski et al., 2010). Paradoxically, the depression of NMDA receptor currents in the parvocellular neurons following acute stress placed the glutamate synapses in a permissive state that allowed them to undergo activity-dependent short-term potentiation in response to high frequency stimulation. Thus, the NMDA receptor mRNA expression and the functionality of the receptor do not appear to be regulated in parallel by stress, which suggests that there may be changes in the receptor protein expression or trafficking that may account for this discrepancy.

Chronic stress plasticity of glutamate inputs

In addition to inducing an apparent functional downregulation in the inhibitory synaptic inputs to PVN parvocellular neuroendocrine cells (Verkuyl et al., 2004), the chronic variable stress model of stress plasticity also gives rise to significant changes in the excitatory synaptic innervation of PVN parvocellular neurons. A double-labeling immunohistochemical study found that chronic variable stress significantly increased the density of synaptophysin-expressing synaptic boutons and VGluT2-expressing glutamatergic fibers in the medial parvocellular PVN, and increased the number of glutamatergic boutons directly contacting CRH-immunoreactive somata and dendrites (Flak et al., 2009). This suggests that chronic stress causes an increase in the glutamatergic synaptic innervation of the CRH neurons, which, along with the reduced GABAergic innervation of medial parvocellular neurons (Verkuyl et al., 2004), should lead to an increase in the excitability of these neurons following chronic stress exposure. Preliminary electrophysiological findings from our laboratory suggest that the increased density of immunolabeled glutamate synapses on the CRH neurons gives rise to an increase in the functional excitatory synaptic inputs to these cells (Franco et al., 2007).

Glutamate receptor expression in the PVN has also been reported to be modified by chronic stress exposure. An in situ hybridization study found that 2 weeks of chronic variable stress caused a decrease in the mRNA expression of the NMDA receptor subunit GluN2B in the medial parvocellular PVN, while no changes in GluN1 or GluN2A subunit expression (Ziegler et al., 2003). This effect differed from the glutamate receptor plasticity induced by acute immobilization stress, which caused an increase in the expression of GluN1 reported in the aforementioned study (Bartanusz et al., 1995). The fact that the decrease in GluN2B induction caused by chronic stress was not accompanied by a change in GluN1 expression suggests that the downregulated
GluN2B does not reflect a decrease in the number of functional NMDA receptors, since the GluN2 subunit is an essential component of functional NMDA receptors (Cull-Candy et al., 2001). The decrease in NMDA receptor mRNA expression with chronic stress is surprising given the observation of an increase in the density of glutamate synapses with the same chronic stress paradigm (Flaks et al., 2009). This suggests either that NMDA receptor mRNA and protein expression are differentially modulated, or that there is less of a contribution of NMDA receptors relative to AMPA receptors at glutamate synapses on CRH neurons following chronic variable stress. Follow-up electrophysiological studies will be necessary to distinguish between these possibilities. Interestingly, the stress-induced downregulation of GluN2B mRNA is apparently not mediated by glucocorticoids, as neither adrenalcorticotropin nor high-dose glucocorticoid administration produced any change in GluN2B mRNA expression in the medial parvocellular PVN (Ziegler et al., 2003). Thus, this plasticity of NMDA receptor expression following chronic variable stress may result from repeated activation of glutamatergic receptors with the chronic activation of stress circuits.

Interestingly, glutamatergic synaptic plasticity resulting from the neonatal environment might be involved in lifelong changes in HPA responsivity. Pups that are handled daily during the first 10 days of life have been found to exhibit a suppressed HPA response to a novel stressor as adults (Liu et al., 1997). It is possible that this effect of handling is mediated by an increase in maternal attention, as handled pups receive more frequent bouts of maternal care in the form of licking, grooming, and arching back nursing. One study using an early-life handling paradigm found that handled pups have lower VGLUT2 protein content in the PVN, fewer VGLUT2-immunoreactive synaptic boutons contacting CRH cells, and fewer asymmetrical, putative excitatory, synapses on CRH cells (Kossos et al., 2010). Electrophysiological recordings in putative parvocellular PVN neurons from these animals found that the frequency of miniature EPSCs also was decreased, suggesting a reduced excitatory synaptic input to the CRH cells. The reduced glutamatergic input in this study was found exclusively in the pups and not following maturation to adulthood, but the handled animals exhibited life-long reductions in PVN CRH expression, which suggests that reductions in glutamatergic input early in life may be involved in a cascade of events that leads to a life-long reduction in CRH expression and HPA stress responsivity.

**NOREPINEPHRINE INPUTS**

Noradrenergic input to the medial parvocellular PVN originates in the brainstem, particularly in the nucleus of the solitary tract (NTS; Herman et al., 2003). Noradrenaline has long been known to play a prominent role in the regulation of the HPA axis and has been shown to be generally excitatory with regard to modulation of CRH neuron activity (Plotky et al., 1987). Accordingly, microinjection of norepinephrine directly into the PVN increases CRH mRNA expression and elevates circulating ACTH in a dose-dependent manner (Isa et al., 1994). There has been debate, however, regarding the role of the brainstem in driving HPA responses to stress. It has been proposed that the brainstem drives HPA responses to systemic stress, whereas HPA responses to psychological stress are driven by regions in the forebrain (Li et al., 1996). In support of this division, the glucocorticoid response to immune stress caused by intraperitoneal injection of interleukin-1α was found to be attenuated by depletion of noradrenergic inputs to the PVN, whereas no such effect of the depleted inputs was observed on the HPA responses to restraint stress (Chalbyan et al., 1992) or footshock stress (List et al., 1996). However, later findings would suggest that ascending noradrenergic signaling plays a role in driving HPA responses to both systemic and psychological stress (Paucal et al., 1998; Days et al., 2003). More recently, Herman et al. (2003) presented an alternative model regarding the role of the brainstem in HPA responses to stress. According to this model, the extent to which the NTS promotes HPA activity results from an interaction between ascending sensory inputs to the NTS and descending inputs to the NTS from the forebrain. It was proposed that the ascending input to the NTS transmits information regarding the current homeostatic state, whereas descending input to the NTS transmits information regarding an anticipated homeostatic state. As a result of this convergence of signaling in the NTS, the HPA response to an anticipated homeostatic state would depend on the current homeostatic state, so that inhibitory signaling from the forebrain can be overridden by current somatic, visceral, or humoral disturbances; alternatively, excitatory signaling from the periphery to the paraventricular PVN could be modulated by descending contextual information (Herman et al., 2003).

Electrophysiological analyses have revealed mixed effects of norepinephrine on putative parvocellular neuroendocrine cells of the PVN; some cells are excited and other cells are inhibited by norepinephrine (Xang et al., 2007). The excitatory actions of norepinephrine on CRH cells appear largely to be mediated by modulation of glutamatergic synaptic inputs. Thus, the surge in circulating ACTH and glucocorticoids resulting from electrical stimulation of the ventral noradrenergic bundle was found to be inhibited by intra-PVN microinjection of ionotropic glutamate receptor antagonists in a dose-dependent manner (Feldman and Weidenfeld, 2004). An electrophysiological study found that about 36% of putative parvocellular neurons responded to norepinephrine with an increase in glutamatergic input, while another 14% responded with a hyperpolarization (Daflary et al., 2000). Post-recording biocytin labeling revealed that the cells that had exhibited a norepinephrine-induced hyperpolarization tended to be located close to the third ventricle in a region known to contain dopamine and/or somatostatin parvocellular neurons, so it is possible that none of the hyperpolarized neurons were CRH cells. The norepinephrine-induced increase in glutamatergic activity was mediated by α2-adrenoceptors and was spike-dependent. Perhaps the most surprising result from our electrophysiological studies is the absence of putative parvocellular neurons that are excited by norepinephrine directly (Daflary et al., 2000). Electron microscopy has revealed the presence of adrenergic boutons forming synaptic specializations with CRH neurons (Upson et al., 1986) and dual in situ hybridization has demonstrated that the α2-adrenoceptor is expressed by nearly all the CRH neurons of the PVN (Day et al., 1999). However, we have found no evidence, and there have been no electrophysiological reports, of norepinephrine directly activating an inward current or depolarization
in parvocellular neuroendocrine cells. As noradrenergic receptors are metabotropic, noradrenergic receptors on CRH cells may initiate saltatory transduction pathways without altering membrane conductance. On the other hand, postsynaptic noradrenergic receptors may stimulate the synthesis of a retrograde messenger that could trigger the activation of upstream local glutamatergic circuits. We have preliminary evidence for just such a retrograde mechanism, in which a messenger is released from CRH neurons in the PVN and stimulates a spike-dependent increase in glutamatergic inputs to the CRH neurons (Jiang and Tasker, unpublished observation, Figure 2).

In addition to regulating glutamate release, norepinephrine modulates GABA release onto parvocellular neurons in the PVN (Figure 2). An electrophysiological study found that about 58% of putative parvocellular neurons responded to norepinephrine with an increase in the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) that was mediated by α2- adrenoceptors and was spike-dependent, suggestive of a locus of action upstream of the GABAergic axon (Han et al., 2002). In another 33% of the neurons tested, norepinephrine reduced the IPSC frequency, and this reduction was mediated by α2- adrenoceptors and was spike-independent, suggesting a presynaptic terminal locus of action (Han et al., 2002). In another study that specifically targeted parvocellular neuroendocrine cells with electrophysiological markers and retrograde staining from the median eminence, norepinephrine suppressed GABAergic input in 46% of the cells tested, which was increased to 100% following blockade of either α2- adrenoceptors or spike generation (Yang et al., 2008). Consistent with α2- adrenoceptors located on GABAergic boutons inhibiting GABA release onto CRH cells, binding assays have reported substantial binding of radiolabeled clonidine, an α2-adrenoceptor agonist, in the CRH cell region of the median paraventricular PVN (Cummings and Seybold, 1988). Moreover, electrophysiological analyses indicate that the α2-adrenoceptor-mediated decrease in GABA input is reflective of a reduced probability of GABA release (Yang et al., 2008). In keeping with its suppressive effect on neurotransmitter release, the α2-adrenoceptor is commonly found to suppress norepinephrine release as an autoreceptor (Batteri et al., 1992). In support of a stimulatory effect of the α2-adrenoceptor on the HPA axis, clonidine has been reported to enhance CRH secretion from organotypic hypothalamic cultures (Calogero et al., 1988), and intracerebroventricular (Staszczuk et al., 1990) and intraperitoneal injections of clonidine in vivo induce a surge in ACTH release.

PLASTICITY OF NOREPINEPHRINE INPUTS

Soon after the discovery that norepinephrine regulates HPA activity, it was found that ascending noradrenergic signaling plays a role in the glucocorticoid-induced suppression of the HPA axis, since depletion of noradrenergic inputs via knife cuts to the medulla attenuated the increases in hypothalamic CRH expression and CRH release caused by adrenalectomy (Sawchenko, 1988). Adrenalectomy was also found to reduce binding of radiolabeled clonidine in the CRH-expressing region of the median paraventricular PVN (Cummings and Seybold, 1988), while α2- adrenoceptor expression in the PVN was upregulated following adrenalectomy (Day et al., 1999). Microdialysis analyses revealed that adrenalectomy increases the synthesis, release, and turnover of norepinephrine in the PVN (Pacak et al., 1993), whereas 1 week of hypercortisolemia, induced by administration of exogenous glucocorticoid, reduced the synthesis, release, and turnover of norepinephrine in the PVN (Pacak et al., 1995). This last finding is surprising inasmuch as 1 week of chronic variable stress has been found to increase both the density of noradrenergic synaptic boutons in the PVN and the number of noradrenergic boutons abutting CRH-immunoreactive somata and dendrites (Fah et al., 2009). Additionally, 1 week of repeated intermittent cold stress was reported to enhance HPA responsivity to an acute immobilization stress via an increase in α1-adrenoceptor signaling within the PVN (Ma and Morilak, 2002).

In addition to the effects of adrenalectomy on norepinephrine release and adrenoceptor expression in the PVN, electrophysiological recordings revealed that adrenalectomy results in an increased fraction of putative parvocellular neuroendocrine cells that are excited by norepinephrine and a decreased fraction inhibited by norepinephrine (Yang et al., 2007). Additionally, it was found that the fraction of cells in which norepinephrine reduced GABA input was doubled following adrenalectomy, whereas there was an 80% decrease in the fraction of cells in which norepinephrine enhanced GABA input (Yang et al., 2008).

Although norepinephrine is thought to be generally excitatory with regard to CRH release, there is a body of evidence that suggests that norepinephrine suppresses CRH neuron activity in the absence of glucocorticoids: thus noradrenergic regulation of CRH cells may be more complex than previously thought. While norepinephrine normally enhances CRH secretion from organotypic hypothalamic cultures, it was found to reduce basal secretion of CRH from cultures maintained in a glucocorticoid-free medium (Staszczuk et al., 1995). Also, while the α2-adrenoceptor agonist clonidine normally promotes CRH cell activity (Calogero et al., 1988), it was found to reduce basal CRH release from organotypic hypothalamic cultures maintained in a glucocorticoid-free medium (Feuvrier et al., 1998). If noradrenergic signaling from the brainstem does in fact play a more prominent role in driving HPA responses to systemic stress than to psychological stress, adrenalectomized rats might be expected to respond differently to certain stressors than do intact rats.

PERPECTIVES AND OUTLOOK

Plasticity of the neural circuitry regulating the HPA axis may constitute an etiological link between stress exposure and the subsequent development of HPA dysregulation and associated pathologies. Research into dysregulation of the HPA axis has focused mainly on hypersecretion of glucocorticoids, which is a condition that has been associated with depressive illness. Autopsies of depressed patients have reported higher than normal levels of CRH and VP mRNA in the PVN (Raadsheer et al., 1994, 1995), which is suggestive of elevated pre-mortem CRH cell activity. It is believed that diminished glucocorticoid-induced suppression of the HPA axis is an underlying factor in the HPA hyperactivity found in depressive illness (Calogero and Nemeroff, 2005). Consistent with this, depressed patients exhibit an attenuated suppression of glucocorticoid secretion when given the...
ACTH response to metyrapone in depressed patients has been observed as a syndrome (Van Houdenhove et al., 2009) have both been linked to issues of hypothalamic-pituitary-adrenal (HPA) axis suppression. With regard to this last phenomenon, it cannot be explained by diminished glucocorticoid activity, and this is at times, been linked to hypoactivity of the HPA axis. Stress, and (3) stress exposure and associated pathologies have, in the past been suggested as contributing factors to the development of subsequent HPA dysregulation and associated abnormalities. To be clear, we refer to the central neural circuitry that governs the HPA axis, thereby mediating glucocorticoid-induced feedback inhibition of the HPA axis. This, however, proposes that a central drive underlies, at least in part, the link between exposure to stress and the subsequent development of HPA abnormalities. There is evidence that enhanced central drive of the HPA axis contributes to the hypercortisolemia associated with depressive illness. Specifically, dexamethasone-resistant depressed patients exhibit an enhanced ACTH response to metyrapone, a glucocorticoid synthesis inhibitor (Fava et al., 1984; Ur et al., 1992). This observation is consistent with a hyperactive drive of the HPA axis that is, in fact, suppressed by glucocorticoids. This glucocorticoid-independent overdrive of the HPA axis may reflect neural circuitry that favors CRH cell hyperactivity. Interestingly, an enhanced ACTH response to metyrapone in depressed patients has been correlated with a reduced efficacy of the selective serotonin reuptake inhibitor fluoxetine for the relief of depressive symptoms (Young et al., 2004), which suggests that resetting to a normal level of central drive to the CRH neurons may prove an effective therapeutic option in cases of depression that are resistant to serotonin-based antidepressants.

Stress- and glucocorticoid-induced plasticity of neural circuitry regulating the HPA axis may constitute a means through which exposure to stress could play a role in a spectrum of HPA abnormalities, including aberrant HPA circadian rhythms, abnormalities in HPA responsivity to stress, and basal HPA dysregulation. We hypothesize that this synaptic plasticity constitutes aetiologic link between exposure to stress and a range of affective and stress-related disorders. We frame this hypothesis on the basis of three premises: (1) exposure to stress increases the likelihood of developing subsequent HPA dysregulation and associated pathologies, (2) rodent models have demonstrated that the synaptic mechanisms that regulate the HPA axis are highly plastic, and that synaptic plasticity is induced by both glucocorticoids and stress, and (3) stress exposure and associated pathologies have, at times, been linked to hypoactivity of the HPA axis, and this phenomenon cannot be explained by diminished glucocorticoid-induced suppression of the HPA axis. With regard to this last premise, fibromyalgia (Tarttervedt et al., 2007) and chronic fatigue syndrome (Van Houdenhove et al., 2009) have both been linked to HPA hyporesponsiveness, and stressful life events have been implicated in the onset of each of these disorders (Hatcher and House, 2005; Gupta and Silman, 2004). There is also evidence that HPA hyporesponsiveness is involved in some cases of post-traumatic stress disorder (PTSD; Yehuda, 2006) and depression (Murphy et al., 1985; Penninx et al., 2007; Ahrens et al., 2008). Sexually abused girls (ages 5–7 years) have exhibited reduced salivary glucocorticoid levels within months following an abusive incident (King et al., 2001). Moreover, adult women who were victims of childhood sexual abuse have exhibited enhanced suppression of glucocorticoids in response to the dexamethasone suppression test (Stein et al., 1997). Lastly, in rats, stress can result in HPA hyporesponsiveness under certain circumstances. One study using female rats found that exposure to intense footshock, followed by 3 weekly reminders of the stimulus, resulted in lower glucocorticoid levels than those recorded previous to the footshock (Louvart et al., 2005). Reminders of the footshock involved placing the rat in a compartment adjacent to the compartment where the footshock had previously occurred, in what was intended as a rodent model of PTSD. Another study found that rats subjected to chronic variable stress, followed by several days recovery from the stress, exhibit attenuated pituitary–adrenal responses to acute novel environment and restraint stresses (Ostrander et al., 2006). It should be noted that there are some mechanisms through which glucocorticoids could actually enhance HPA activity. For example, glucocorticoids seem to attenuate GABAergic restraint on CRH cells, as described above; thus, in certain cell types, downregulated GR expression in response to stress could promote subsequent HPA overactivity. However, there is evidence that stress can, at times, result in upregulated GR. GR number was found to be larger in lymphocytes of combat veterans than in those of non-veterans (Yehuda et al., 1995). Also, increased GR binding capacity has been reported in the rat hippocampus following repeated inescapable footshock stress (van Dijken et al., 1993). Moreover, the enhanced response to the dexamethasone suppression test in adult victims of childhood sexual abuse (Stein et al., 1997) is suggestive of an increased sensitivity to glucocorticoids, at least at the anterior pituitary (Cole et al., 2000). Upreregulated GR expression could be a protective response to hypocortisolemia, as some degree of GR plasticity that promotes CRH cell suppression. Indeed, GCR expression generally increases following adrenalectomy (Uple and McEwen, 1976; Svec et al., 1989; Isenovic et al., 2006).

With respect to future investigations of neural regulation of the HPA axis, recent advances in circuit tracing technology have seen the development of a Cre-dependent retrogradely transported marker derived from the rabies virus that will allow investigators to trace the precise sources of innervation to CRH cells (Woll et al., 2010). This tool will inevitably improve our understanding of how the brain integrates pertinent information into an HPA response. Also, one investigative team recently reported that peri- natal stress, caused by an active construction site adjacent to the rodent vivarium, resulted in hypocortisolemia that persisted even after the construction had ended (O’Regan et al., 2010), which underscores the sensitivity of HPA regulatory mechanisms to environmental influences (at least perinatally), but also serves as a warning to investigators of the importance of documenting the

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living conditions under which their subjects are kept. Lastly, it is important that assays of morphological plasticity and changes in ionotrophic receptor expression are accompanied by electrophysiological recordings, as electrophysiology provides invaluable information regarding functional manifestations of synaptic activity. Dysregulation of the HPA axis may be a causal factor in a range of affective and physiological disorders, and there is increasing evidence that stress can result in persistent changes in HPA regulation. Rodent models of the past several years have provided significant insight into the etiology of stress-induced aberrations in HPA activity, but our understanding is still in its infancy. Studies of synaptic transmission will remain invaluable to this provocative and promising line of research, as we uncover future targets for pharmacological intervention.

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