Neurocircuitry of Stress Integration: Anatomical Pathways Regulating the Hypothalamo-Pituitary-Adrenocortical Axis of the Rat

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SYNOPSIS. The hypothalmo-pituitary-adrenocortical (HPA) axis is recruited by the organism in response to real or perceived threats to homeostasis (“stress”). Regulation of this neuroendocrine system is accomplished by modulation of secretory tone in hypophysiotrophic neurons of the medial parvocellular paraventricular nucleus. Excitation of these neurons is mediated by several sources: direct (and perhaps indirect) inputs from brainstem neurons regulating autonomic tone/arousal; circumventricular organs monitoring blood and CSF constituents; and local-circuit neurons within the hypothalamus and basal forebrain. The latter are predominantly GABAergic; notably, these areas are targets for descending GABAergic input from limbic structures, and may promote PVN secretory activity via disinhibition. Neurosecretory paraventricular nucleus neurons are inhibited by glucocorticoid-dependent and independent mechanisms. Glucocorticoid negative feedback appears to act both locally and in extrahypothalamic loci, and is likely integrated in a region- and stressor-specific manner. Inhibitory input to the medial parvocellular paraventricular nucleus emanate predominantly from the bed nucleus of the stria terminalis and hypothalamus, and are likely regulated by neuroendocrine homeostats. Descending limbic inhibitory information appears to act through excitation of these inhibitory inputs. Overall, integration of stressful information is a multi-faceted process integrating prior experience and real or anticipated homeostatic disruption into appropriate activation and deactivation of the hypothalamo-pituitary-adrenocortical axis.

The hypothalmo-pituitary-adrenocortical (HPA) axis is required for adaptation of organisms to changes in the internal or external environment. Glucocorticoids, the end-product of the HPA axis, promote survival-enhancing physiologic changes via their activation of classical steroid receptor mediated transcriptional regulation and, possibly, membrane receptors. In general, glucocorticoid-induced changes serve to mobilize energy and inhibit other potentially costly reactions to stress. Thus, glucocorticoids promote catabolism, increase blood pressure, suppress aspects of immune and reproductive function, and inhibit synthesis and secretion of other endocrine hormones (McEwen and Stellar, 1993).

Given the powerful catabolic effects of glucocorticoids on the organism, it is critical that the magnitude and duration of the glucocorticoid response to stress is limited and proportionate to the context of any stressful challenge. This control is accomplished by a “glucocorticoid negative feedback” loop, whereby the steroids can limit their own release and thereby terminate responses efficaciously (Keller-Wood and Dallman, 1984). “Feedback” appears to be controlled primarily by the brain (Levin et al., 1988); however, as we shall see, there is probably not a single locus in brain that subserves this function. Limitation of the stress response is clearly a priority; all vertebrate organisms tested display a feedback response (Butler et al., 1969; Callard et al., 1975; Laub et al., 1975; As- theimer et al., 1994), and disruption of feedback can result in cumulative, deleterious effects of glucocorticoids on the body and brain.

Thus, adequate control of the glucocorticoid stress response is a major priority for all organisms. Adaptation is required for the reproductive success and perpetuation of all species, and the HPA system has been selected and maintained through vertebrate phylogeny as a hormonal defense against changes in the external or internal environment. In the present review, we will present a brief summary of the literature on organization of central HPA projections, and present our current perspective on neurocircuit regulation of PVN neuronal activation.

THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL AXIS

The HPA axis is a classic neuroendocrine loop (see Fig. 1). Glucocorticoid secretion is initiated by a discrete set of neurosecretory neurons located in the medial parvocellular paraventricular nucleus (PVN) (Antoni, 1986; Whitnall, 1993). These PVN neurons synthesize a number of ACTH secretagogues, the most important of which are corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) (Antoni, 1986; Whitnall, 1993). Notably, CRH and AVP have synergistic effects on pituitary ACTH release (Gillies et al., 1982), and as such, AVP is thought of as an amplifier of the stress response. When released, ACTH is transported by the systemic circulation to the adrenal cortex, where it promotes glucocorticoid synthesis and secretion.

Parvocellular PVN CRH/AVP neurons are the motorneurons of the stress response (Antoni, 1986; Whit-
an interface between higher brain centers and PVN activation, serving as the gateway through which multimodal sensory information and stored memories elaborate stress responses. The combinatorial properties of these three systems likely dictate the strength and salience of individual stressors.

Brainstem pathways

Activity of the brainstem is integral to autonomic function, somatosensory/pain processing, and mediating arousal. As such, it would be logical for various brainstem loci to promote HPA activation, and this indeed appears to be the case.

The medial parvocellular PVN is in direct receipt of information from catecholaminergic as well as non-catecholaminergic regions of the nucleus of the solitary tract (ter Horst and Luiten, 1987; Cunningham and Sawchenko, 1988; Sawchenko et al., 1988). Accordingly, catecholaminergic pathways neurons appear to be critical to HPA induction following stress (Plotsky et al., 1989); for example, local administration of norepinephrine or stimulation of ascending noradrenergic pathways stimulates CRH release in an alpha-adrenoreceptor dependent fashion (Plotsky, 1987; Szafarczyk et al., 1987). Likewise, damage to ascending norepinephrine pathways attenuates HPA activation following stress (Szafarczyk et al., 1987), and microdialysis studies confirm release of norepinephrine in the PVN region following a variety of stressful stimuli (Pacak et al., 1995). The ascending NE pathways appear to differentially affect HPA responsiveness as a function of the nature of stressful stimuli; for example, damage to the ventral noradrenergic bundle inhibits CORT responses to ether but not restraint (Gaillet et al., 1991; Herman et al., 2002), and removal of ascending medullary projections block PVN neuronal activation, as measured by cFos induction, following interleukin-1β injection but not footshock (Li et al., 1996). The A2 region is known to relay information on cardiovascular tone and blood oxygenation directly to the PVN (ter Horst et al., 1989; Cunningham et al., 1990); as such, this area is in position to provide a short-latency signal to the PVN, rapidly initiating glucocorticoid responses to aid in the defense against cardiovascular collapse. However, it is clear that this region may also contribute to forebrain regulation of HPA activity. For example, damage to the central amygdaloid nucleus reduces activation of NTS and PVN neurons following IL-1β injections (Xu et al., 1999) and attenuates stress-induced norepinephrine release in the PVN (Beaulieu et al., 1987). Given the lack of a direct input to the medial parvocellular PVN (Table 2), these data suggest that CeA modulation of HPA activity may occur via its projection to the NTS (Schwaber et al., 1982).

The locus coeruleus, another ascending noradrenergic pathway, is a key system involved in arousal and plays a secondary yet important role in HPA activation. Lesions of the locus coeruleus reduce stress-induced CORT secretion following restraint, indicating

Fig. 1. Schematic diagram of the hypothalamo-pituitary-adrenocortical axis. Glucocorticoid secretion is initiated by parvocellular corticotropin-releasing hormone and vasopressin containing neurons of the paraventricular nucleus of the hypothalamus. Following activation by central stimulatory circuitry (see Fig. 2), CRH and AVP are released into the pituitary portal circulation at the median eminence (ME). Secretagogues induce release of ACTH at the level of the anterior pituitary. ACTH then promotes synthesis and secretion of glucocorticoids at the level of the adrenal cortex. Glucocorticoids exert direct and transsynaptic negative feedback inhibition of the hypothalamic-pituitary-adrenal (HPA) axis at multiple sites in the brain. Figure is reprinted from (Herman et al., 1996), with permission.
Table 1. Projections to the medial parvocellular PVN.

<table>
<thead>
<tr>
<th>Region</th>
<th>Neurotransmitter</th>
<th>Innervation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brainstem</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laterodorsal Tegmental N./Pedunculopontine N.</td>
<td>ACh</td>
<td>sparse</td>
</tr>
<tr>
<td>Periaqueductal Gray</td>
<td>5-HT</td>
<td>sparse</td>
</tr>
<tr>
<td>Raphé N. (B7, B8, B9)</td>
<td>NE</td>
<td>dense</td>
</tr>
<tr>
<td>C2 region</td>
<td>E</td>
<td>dense</td>
</tr>
<tr>
<td><strong>Circumventricular/lamina terminalis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfornical Organ</td>
<td>Angiotensin II</td>
<td>very dense</td>
</tr>
<tr>
<td>OVLT</td>
<td></td>
<td>moderate-dense</td>
</tr>
<tr>
<td>Median Preoptic Area</td>
<td></td>
<td>very dense</td>
</tr>
<tr>
<td><strong>Local Circuits (Hypothalamus)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Preoptic N.</td>
<td>GABA, neuropeptides</td>
<td>moderate</td>
</tr>
<tr>
<td>Lateral Preoptic N.</td>
<td>GABA, neuropeptides</td>
<td>moderate</td>
</tr>
<tr>
<td>Anteroventral Periventricular N.</td>
<td>GABA, neuropeptides</td>
<td>moderate</td>
</tr>
<tr>
<td>Subparaventricular Zone</td>
<td>GABA</td>
<td>moderate-dense</td>
</tr>
<tr>
<td>Perifornical N.</td>
<td>GABA</td>
<td>moderate</td>
</tr>
<tr>
<td>Lateral Hypothalamic Area</td>
<td>GABA, neuropeptides</td>
<td>moderate</td>
</tr>
<tr>
<td>Dorsomedial Nucleus</td>
<td>GABA, glutamate</td>
<td>moderate</td>
</tr>
<tr>
<td>Arcuate Nucleus</td>
<td>GABA, neuropeptides</td>
<td>moderate</td>
</tr>
<tr>
<td>Posterior Hypothalamic Area</td>
<td></td>
<td>moderate</td>
</tr>
<tr>
<td>Ventral Premamillary N.</td>
<td></td>
<td>moderate-sparse</td>
</tr>
<tr>
<td>Supramamillary N.</td>
<td></td>
<td>moderate</td>
</tr>
<tr>
<td><strong>Basal Forebrain Circuits</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior Medial BST</td>
<td>GABA</td>
<td>moderate-dense</td>
</tr>
<tr>
<td>Posterior Intermediate BST</td>
<td>GABA</td>
<td>moderate-dense</td>
</tr>
<tr>
<td>Posterior Lateral BST</td>
<td>GABA</td>
<td>sparse</td>
</tr>
<tr>
<td>Ventral Medial BST</td>
<td>GABA</td>
<td>moderate</td>
</tr>
<tr>
<td>Anterior Medial BST</td>
<td>GABA</td>
<td>moderate</td>
</tr>
<tr>
<td>Anterior Dorsal BST</td>
<td>GABA</td>
<td>sparse</td>
</tr>
<tr>
<td>Ventromedial Zona Incerta</td>
<td>GABA, DA</td>
<td>dense</td>
</tr>
<tr>
<td>V. Substantia Inominata</td>
<td>where known</td>
<td>moderate</td>
</tr>
</tbody>
</table>

Neurotransmitter reflects the likely phenotype of the projection; innervation refers to density of fiber plexus in the medial parvocellular PVN and/or approximate density of retrogradely labeled neurons in PVN-projecting nuclei. References for this table were from the following, as well as observations in our laboratory:

a role in stress activation (Ziegler et al., 1999). However, the locus coeruleus does not appear to have direct projections to the PVN (Cunningham and Sawchenko, 1988), indicating that modulation of HPA activation is trans-synaptic. Importantly, the locus coeruleus projects to forebrain regions that modulate CORT secretion, including the amygdala, prefrontal cortex and hippocampus (Grant and Redmond, 1981), providing a mechanism whereby this important attentional system may interface with HPA-regulatory circuitry.

Both serotonin (5-HT) and acetylcholine have excitatory effects on HPA axis activation in vivo (Feldman et al., 1987, 1991; Plotsky et al., 1987; Saphier and Feldman, 1989; Ohmori et al., 1995) and in vitro (Grossman et al., 1993). Interestingly, 5-HT and acetylcholine innervation of the PVN is relatively sparse; the parvocellular region contains a very modest 5-HT input (Sawchenko et al., 1983; Larsen et al., 1996), and few if any choline acetyltransferase positive fibers (Mesulam et al., 1983; Hallanger and Wainer, 1988). In both cases the innervation of other hypothalamic, thalamic, and telencephalic regions far surpasses that seen in the PVN. Nonetheless, both neurotransmitters promote CRH, ACTH and/or CORT release when directly applied to the region of the PVN or to PVN containing explant cultures (Jones and Gillham, 1988; Grossman et al., 1993; Ohmori et al., 1995; Jorgensen et al., 1998). As with the locus coeruleus, both systems play major roles in mediating global arousal or facilitating attention to alerting stimuli; thus, the ability of these systems to evoke CORT secretion is logical. The anatomical pathways underlying action of these transmitters are unclear, and may involve action of the few direct projections (from the serotonergic raphe nuclei and cholinergic lateral dorsal tegmental and pedunculopontine nuclei) (Mesulam et al., 1983; Sawchenko et al., 1983; Hallanger and Wainer, 1988; Larsen et al., 1996) or interactions with local-circuit interneurons.

Ascending pain pathways provide a powerful stimulatory input to the HPA axis. The work of Palkovits and colleagues has provided a detailed analysis of ascending noradrenergic pathways communicating painful information to the PVN. These travel via noradrenergic neurons of the ventrolateral medulla, but may also involve the A2 region or locus coeruleus (Palkovits et al., 1999).

Overall, the ascending brainstem pathways allow for rapid, often direct transmission of interoceptive, arousal, and pain information to the PVN. Furthermore, these pathways may also be co-opted by descending information from limbic/autonomic structures, allowing brainstem signaling to participate in trans-synaptic HPA activation as well.

Circumventricular organs

The medial parvocellular PVN receives a rich innervation from the circumventricular organs, most notably the subfornical organ (SFO) and to a lesser extent, organum vasculosum of the lamina terminalis (OVLT). Projections from the SFO are angiotensinergic and promote CRH secretion and biosynthesis (Lind et al., 1984; Plotsky et al., 1988; Aguilera et al., 1995). This pathway likely mediates the effect of osmotic stress on HPA activation (Kovacs and Sawchenko, 1993).

The SFO is an important component of the lamina terminalis system of neurons that also encompasses the OVLT and median preoptic nucleus. This aggregation of neurons is believed to form a major component of circuitry regulating fluid and electrolyte balance and drinking behavior (McKinley et al., 1996). This projection pathway has parallel input to the magnocellular neurosecretory system (Lind et al., 1984), and likely serves to link HPA and neurohypophysial activation during periods of osmotic imbalance.

Other CVOs may be indirectly involved in HPA in-

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**Table 2. Projections to the peri-PVN region.**

<table>
<thead>
<tr>
<th>Region</th>
<th>Neurotransmitter</th>
<th>Innervation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral Subiculum</td>
<td>Glutamate</td>
<td>dense</td>
</tr>
<tr>
<td>Medical Prefrontal (Infralimbic)</td>
<td>Glutamate</td>
<td>moderate</td>
</tr>
<tr>
<td>Medial Amygdaloid N.</td>
<td>GABA, neuropeptides</td>
<td>moderate</td>
</tr>
<tr>
<td>Ventrolateral Septum</td>
<td>GABA</td>
<td>moderate-dense</td>
</tr>
<tr>
<td>Suprachiasmatic N.</td>
<td>GABA, neuropeptides</td>
<td>dense</td>
</tr>
<tr>
<td>Ventromedial Hypothalamic N.</td>
<td>GABA</td>
<td>dense</td>
</tr>
</tbody>
</table>

See Table 1 for references.

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**Table 3. PVN afferents with minimal input to the medial parvocellular region.**

<table>
<thead>
<tr>
<th>Region</th>
<th>Neurotransmitter</th>
<th>PVN region innervated</th>
<th>Innervation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Amygdaloid n.</td>
<td>GABA, neuropeptides</td>
<td>dorsal, lateral parvocellular div.</td>
<td>sparse</td>
</tr>
<tr>
<td>Dorsal lateral BST</td>
<td>GABA, neuropeptides</td>
<td>dorsal, lateral parvocellular div.</td>
<td>sparse</td>
</tr>
<tr>
<td>Suprachiasmatic n.</td>
<td>GABA, neuropeptides</td>
<td>dorsal, lateral parvocellular div.</td>
<td>moderate</td>
</tr>
<tr>
<td>Lateral parabrachial n.</td>
<td>Neuropeptides</td>
<td>periventricular zone</td>
<td>moderate</td>
</tr>
<tr>
<td>Locus Coeruleus</td>
<td>NE</td>
<td>magnocellular divisions</td>
<td>dense</td>
</tr>
<tr>
<td>Al/C1 region</td>
<td>NE, E</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See Table 1 for references.
tegration. The medullary area postrema does not have a substantial direct projection to the PVN (Oldfield and McKinley, 1995). However, damage to the area postrema markedly impairs PVN cFos activation and ACTH secretion following systemic injection of IL1β (Lee et al., 1998). Thus, this pathway may serve as an initiation point for cytokine signaling in the CNS, probably through relays in the NTS (see above). However, additional evidence suggests that cytokines may also signal neuronally via the vagus or humorally through interactions with perivascular cells (Ericsson et al., 1994; Ishizuka et al., 1997; Maier et al., 1998; Goehler et al., 1999).

**Basal forebrain and hypothalamic pathways**

Local projections comprise the lion’s share of inputs to the paraventricular PVN. These include prominent inputs from the dorsomedial nucleus, medial preoptic area, anteroventral third ventricular region, and ventral medial, posterior medial and posterior intermediate divisions of the bed nucleus of the stria terminalis (BST). In addition, the PVN receives substantial innervation from cells in the immediate surround of the nucleus, as well as the adjacent subparaventricular zone and perifornical area (Sawchenko and Swanson, 1983; Roland and Sawchenko, 1993). These regions, including the peri-PVN region, are in receipt of descending input from several limbic forebrain sites (Table 2) and are thus likely to gate limbic input proximal to the PVN.

The neurochemistry by which these local afferent sites communicate with the PVN is of a dual nature, mediating both excitation and inhibition of the stress axis (Figs. 2 and 3). Curiously, from the standpoint of elucidating HPA-excitatory circuits, these local projections to the PVN are predominantly GABAergic in phenotype (Cullinan et al., 1993; Roland and Sawchenko, 1993) and are therefore positioned to supply a powerful source of inhibition on basal and stress-induced PVN and HPA axis activity. Indeed this GABAergic neurocircuitry is the focus of the section below on HPA-inhibitory systems in the CNS. However, these PVN-projecting regions receive innervation from upstream stress- or memory-related limbic sites, including the lateral septum and medial amygdala, which also contain substantial populations of GABAergic projection neurons (Risold and Swanson, 1996, 1997). Thus, these more remote regions could participate in bi-synaptic GABA-GABA connections with the PVN, resulting in a disinhibition of HPA activity.

Local circuit excitation of the PVN may also be initiated by glutamatergic afferents, a mechanism suggested by multiple lines of investigation. Parvocellular PVN neurons express both NMDA and kainate receptor subunits (Herman et al., 2000), indicating the capacity to integrate glutamatergic input. Furthermore, approximately half of all synapses on PVN neurons are glutamatergic (Decavel and Van Den Pol, 1992). Electrophysiological studies document a robust excitation of PVN neurons by glutamatergic transmission (Boudaba et al., 1997). This role is supported by pharmacological studies reporting blockade of stress responses by intra-PVN infusion of ionotropic glutamate antagonists (Ziegler and Herman, 2000) or, conversely, activation of the HPA axis by microinfusion of glutamate (Darlington et al., 1989; Feldman and Weidenfeld, 1997). Furthermore, NMDA receptor subunit mRNA expression in the PVN is down-regulated by chronic intermittent stress, but is not regulated by glucocorticoids [Ziegler and Herman, unpublished observations], suggesting that glutamate synapses in the PVN are repeatedly and intensely activated during chronic stress exposure. Whereas evidence for PVN glutamate action is clear, the sources of glutamate innervation remain relatively unexplored. Electrophysiological studies provide evidence for glutamate interneurons in the peri-PVN region and within the PVN itself, suggesting action proximal to the CRH neuron (Daftary et al., 1998). In addition, pharmacological and electrophysiological studies (Tasker et al., 1998) suggest that the dorsomedial hypothalamus and perifornical area may supply glutamate innervation, suggesting that the heavily GABAergic PVN-projecting nuclei of the basal forebrain and hypothalamus also contain small but important subpopulations of neurons providing direct excitatory input to the HPA axis. Excitatory interactions between limbic structures and the PVN may also be effected indirectly through a third mechanism: relay via the brainstem pathways discussed above. The central amygdaloid nucleus (CeA) may well function in such a fashion, as it does not show substantial direct projections to the PVN (Gray et al., 1989; Prewitt and Herman, 1998), and does not appear to connect with the PVN through the hypothalamus or BST (Prewitt and Herman, 1998). Instead, this information appears to relay via brainstem catecholaminergic pathways (Xu et al., 1999). Several other limbic regions (e.g., prefrontal cortex, lateral hypothalamus) (van der Kooy et al., 1984; Zardetto-Smith et al., 1988) have substantial projections to the NTS area and may function in similar fashion.

**Inhibitory Stress Pathways**

**"Glucocorticoid negative feedback" and stress inhibition**

Inhibition of the HPA axis is mediated by humoral (glucocorticoid-mediated) and neuronal (glucocorticoid-independent) pathways (Fig. 3) (Keller-Wood and Dallman, 1984; Herman and Cullinan, 1997). The former comprise part of the so-called "glucocorticoid negative feedback" loop, and serve to inhibit further activation of the HPA axis. The nature of negative feedback is currently obscure. Glucocorticoids were traditionally assumed to exert delayed effects via steroid receptor-mediated genomic action; however, glucocorticoids can inhibit ACTH secretion within minutes (so-called "fast feedback") (Keller-Wood and Dallman, 1984; Young et al., 1990), consistent with
non-genomic regulation by mechanisms yet to be identified.

The anatomical locus of glucocorticoid negative feedback is currently undefined. Hypophysiotropic PVN neurons contain glucocorticoid receptors (Uht et al., 1988), and appear to integrate at least some measure of negative feedback, as dexamethasone (but not corticosterone) implants into the region of the PVN reverse adrenalectomy-induced ACTH hypersecretion, and local application of cortisol reduces PVN neuronal activity (Sawchenko, 1987; Kovacs and Makara, 1988; Saphier and Feldman, 1990). However, total or anterior deafferentation of the PVN increases CRH and AVP mRNA expression (Herman et al., 1990), indicating that extrahypothalamic regions provide inhibitory input to hypophysiotropic neurons. Similarly, anterior hypothalamic lesions reduce the ability of CORT to inhibit adrenalectomy-induced ACTH hypersecretion (Levin et al., 1988). The locus of extra-PVN feedback inhibitory input remains to be determined. Lesion studies implicate the hippocampus, prefrontal cortex and medial preoptic area in inhibition of basal and/or stress induced HPA activation (Jacobson and Sapolsky, 1991; Diorio et al., 1993; Viau and

Fig. 2. **Top.** Overview of the three functional/neuroanatomical classes of excitatory inputs to the PVN/HPA axis. Some pathways have direct projections to the PVN, others are shown connecting via intermediating, local neurons in the basal forebrain and hypothalamus, located within the shaded region. (1) Converging afferents conveying physiological stressors (e.g., hypotension, hypoglycemia, pain, and arousing stimuli): NTS = nucleus of the solitary tract (also containing noradrenergic A2/C2 groups), A1/C1 = ventral medullary NE groups; arousal-related noradrenergic LC (locus coeruleus), serotonergic dorsal raphe, and the cholinergic LDT (lateral dorsal tegmental nucleus) and PPN (pedunculopontine nucleus). Note these arousal systems also project throughout the forebrain (not shown for clarity), thus facilitating transduction of progressive stress (3) as well. (2) Bloodborne signals (e.g., blood osmolarity, cytokines) are conveyed to the PVN via circumventricular organs (CVOs): the SFO (subfornical organ) and OVLT (organum vasculosum of the lamina terminalis). (3) Complex stressors (e.g., social stress, restraint, novelty) are transduced in stages: stimuli are integrated in sensory and association cortex, filtered for significance by the amygdala (AMY, in temporal lobe) lateral septum, and possibly other unidentified structures, which then activate the PVN via connections with intermediary, local regions (shaded region) with direct projections to the PVN. **Bottom.** Schematic representation of the neurochemistry of HPA-excitatory circuitry. CVO projections use angiotensin II (AII). The amygdala and lateral septum signal via two possible mechanisms: glutamate-glutamate or GABA-GABA bisynaptic connections with the PVN. The final PVN-projecting neurons reside in local regions: the basal forebrain (BST), hypothalamic nuclei, and the immediate surrounding peri-PVN zone.
FIG. 3. Top. Neurocircuitry mediating HPA-inhibition. After initial stress transduction by sensory pathways, the significance of this information is evaluated by the medial prefrontal cortex (PFC), hippocampus (HPC) and/or ventral subiculum (vSub), which inhibit the PVN via bysynaptic glutamate-GABA connections (see bottom also), supplying excitatory innervation (filled arrowheads) to three local inhibitory PVN-projecting regions: the BST (bed nuclei of the stria terminalis), hypothalamic nuclei (dark ovals), and to a zone of interneurons (minus signs) immediately surrounding the PVN itself, all of which are GABAergic (open arrowheads).

ac anterior commissures. Bottom. Schematic depiction of this circuitry, which is also responsive to glucocorticoid levels. Glucocorticoid negative feedback may not only act at the PVN and pituitary levels, but also via upstream sites.

Meaney, 1996; Herman and Cullinan, 1997). Notably, all of these regions are rich in glucocorticoid receptors (Herman, 1993), indicating a plausible role in feedback. Consistent with this notion, implants of glucocorticoids into the hippocampus, prefrontal cortex and preoptic area reduce basal and/or restraint-induced ACTH/CORT release (Jacobson and Sapolsky, 1991; Diorio et al., 1993; Viau and Meaney, 1996). However, not all studies support this hypothesis; for example, damage to the primary hippocampal outflow tract (fornix) does not disinhibit hypoxia-induced ACTH release (Bradbury et al., 1993). Moreover, the neuronal glucocorticoid receptor is known to regulate a wide-range of cellular functions, among them a decrease in excitability (and outflow, presumably) following exposure to very high or persistently elevated levels glucocorticoids (Joels and deKloet, 1992). Thus, it remains controversial as to whether this HPA inhibition represents glucocorticoid-independent neural regulation or if the glucocorticoid receptor in these regions confers the additional capacity for extra-PVN negative feedback.

The effusiveness of central negative feedback is inconsistent with an anatomically discrete feedback integrator. The fact that feedback effects can be elicited at multiple sites, and that the glucocorticoid receptor is localized to a wealth of HPA-regulatory regions (including the amygdala, raphe nuclei and all brainstem NE/E subgroups [Gustafsson et al., 1987; Sawchenko and Bohn, 1989; Herman, 1993]) suggests that glucocorticoid information is processed as a pattern. Thus, glucocorticoids are in position to interact with ongoing neurotransmission in individual cell groups activated (or inhibited) by stress. Feedback effects are likely important within the context of the stressful stimulus; if the region in question is not queried by...
the stimulus, the impact of glucocorticoid secretion is irrelevant. This likely explains the fact that hippocampal and prefrontal cortex lesions disrupt effects of novelty/restraint but not ether inhalation or hypoxia (Bradbury et al., 1993; Diiorio et al., 1993; Herman et al., 1998); both regions respond well to spatial and experiential cues, but would not be directly involved in integrating information from interoceptive sensory pathways.

The existence of glucocorticoid-independent stress inhibition (see (Keller-Wood and Dallman, 1984)) indicates that inhibitory afferents to the PVN can function in the absence of glucocorticoids. This is not surprising, as membrane "glucocorticoid" receptor and/or glucocorticoid receptor-mediated genomic signaling represent but one facet of the function of stress-inhibitory neurons. While clearly important, it is no doubt the case that inhibitory innervation can function in the absence of feedback. Glucocorticoids are but one component of the inhibitory process.

Local-circuit neurons: gateway to PVN inhibition

As noted, the PVN receives a rich innervation from the region of the hypothalamus, preoptic area and bed nucleus of the stria terminalis (Sawchenko and Swanson, 1983; Cullinan et al., 1993; Roland and Sawchenko, 1993). This innervation is overwhelmingly GABAergic (Cullinan et al., 1993; Roland and Sawchenko, 1993). Thus, it is reasonable to postulate that the vast majority of local input to the PVN is inhibitory. An inhibitory role for GABA in HPA regulation is supported by the presence of GABA-A receptor subunits in the PVN (Cullinan, 2000).

The rich GABA innervation of the PVN implies a change in valence of descending input at the PVN: inhibition is likely initiated by stimulation of GABA neurons. Notably, combined anterograde-retrograde tract-tracing studies indicate that PVN-projecting GABAergic neurons are asayed by ventral subicular afferents (Cullinan et al., 1993), which are glutamatergic in phenotype (Walaas and Fonnum, 1980). As such, enhanced hippocampal activation during stress can be translated into PVN inhibition by way of this pathway (Herman and Cullinan, 1997). Similar pathways may subserve descending input from other cortical regions, including the prefrontal and cingulate cortices.

The glutamate-GABA circuit hypothesis is supported by data indicating that blockade of ionotropic glutamate receptors in the peri-PVN region enhances HPA stress responses (Ziegler and Herman, 2000). This effect is likely mediated by rich localization of NMDA, AMPA and kainate receptor subunit mRNAs in these regions (Herman et al., 2000). Stress regulatory studies are similarly consistent with this interpretation: PVN-projecting neuronal populations show marked cFos up-regulation following acute restraint, open field and swim stress (Cullinan et al., 1995, 1996; Emmert and Herman, 1999), and show up-regulation of GAD65 mRNA (Bowers et al., 1998) and down-regulation of NMDA-R2 mRNA (Ziegler and Herman, 1995) in response to chronic intermittent stress exposure.

The diversity of intra-hypothalamic PVN projecting pathways implies multiple avenues for HPA regulation. For example, the ventral subiculum and prefrontal cortex proffer parallel projections to numerous hypothalamic PVN-projecting loci (Canetars and Swanson, 1992; Cullinan et al., 1993; Ongur et al., 1998; Kishi et al., 2000). The information from these limbic regions is distributed amongst hypothalamic sites, and it is probable that the net impact on the HPA axis depends on the context in which it is received. Thus, it is possible that activation of intrahypothalamic PVN pathways by homeostats (e.g., hunger, thirst, sex drive) can color the efficacy of upstream limbic effectors, perhaps diminishing the importance of the limbic input in favor of adjusting the HPA axis to cope with physiologic demand.

Importantly, hypothalamic systems subserving feeding homeostatic functions (such as energy balance) comprise the same relay nuclei contacted by descending limbic input. For example, circulating factors such as leptin and insulin invoke projections from the medio-basal hypothalamus (ventromedial nucleus/arcuate nucleus) to the dorsomedial hypothalamus and PVN itself (Baskin et al., 1999). Similarly, cytokines appear to signal via the preoptic area (Saper, 1998). Indeed, the glucocorticoid response is vital for both energy balance and immunity, and the PVN constitutes a target for these important homeostatic systems. Thus, homeostatic disruption may override descending inhibitory input to the PVN via these local circuits.

Stress-specific pathways in brain: complexity of connections, simplicity of function

The net result of the last several years of stress-circuit research has been the discovery of a wealth of stimulus-specific pathways that defy simple classification. Our own work suggested two primary types of stress circuits: brainstem, CVO and hypothalamic circuits relaying information on physiologic state, and a multisynaptic circuitry controlling HPA activation subsequent to assembly of multi-modal sensory information and comparison with past experience. These responses may be viewed as "reactive" vs. "anticipatory": the reactive stress responses occur during a frank physiological challenge (e.g., blood loss, low blood sugar), whereas anticipatory responses are generated to control the net impact of a novel or known stressor. Overlap amongst pathways likely occurs with reactive stressors; the stress response proffered to physiological challenge also encodes information on situation-specific aspects of the stimulus (perhaps through glucocorticoids as well as reciprocal neural interactions). For example, hypoxia may well evoke activation and regulatory responses in descending forebrain stress circuits, by the conscious experience of hypoxia (labored breathing, etc.) and its consequent glucocorticoid surge. Thus, a reactive stress presumably generates a central trace that will affect stress...
responses occurring during re-exposure to the context of the original challenge. As such, it is unlikely that a CNS response to a reactive stressor will occur without parallel activation of pathways encoding contextual information.

Nonetheless it is clear that reactive and proactive response pathways use different CNS substrates. The choice of circuits is likely key to specific sensory and associational response patterns: for example, a spatial stimulus (e.g., novel environment) will use the hippocampus to control the HPA response, whereas a non-spatial stimulus (e.g., hunger, pain) would not require hippocampal input for regulating the HPA input. Recent studies suggest that response patterning may be even further subdivided; for example, cFos activation in proactive stress pathways can differ substantially with the nature of the stimulus, and indeed can differentiate individuals placed in the same evocative situation (e.g., social interaction, learned helplessness paradigms) (Kollack-Walker et al., 1997; Steciuk et al., 1999). The involvement of a given brain structures in control of stress responses to such stimuli is dependent on its role in interpretation of the stimulus and the individual response predispositions of the organism.

Finally, it is important to point out that the subcortical interface between the limbic system and the PVN lies within the path of homeostatic signaling processes. Thus, an opportunity is presented for homeostatic signals to preside over anticipatory response pathways. Such an arrangement makes heuristic sense, as systemic stimuli such as blood loss, hypoglycemia or inflammation provide a signal to which the organism must respond to survive. The gating of limbic information through such structures provides a mechanism whereby the physiologic stimulus can override or ignore the psychological one, and thus assure that a stress response is not inhibited or impaired by upstream input when glucocorticoid secretion is required. By similar logic, the upstream effectors may interface with the ongoing activity of homeostatic systems, and lead to dysregulation in disease states associated with dysfunctional stress integration.

In summary, a diversity of CNS systems are engaged in transducing, monitoring and evaluating the internal and external environment and are responsible for integrating multimodal, sometimes conflicting, information. In this way, HPA axis function may be optimized in accordance with the nature of stressful challenges as well as with prior experience, motivational state and the recent history of glucocorticoid secretion.

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