Neurocircuitry of stress: central control of the hypothalamo–pituitary–adrenocortical axis

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Integration of the hypothalamo–pituitary–adrenal stress response occurs by way of interactions between stress-sensitive brain circuitry and neuroendocrine neurons of the hypothalamic paraventricular nucleus (PVN). Stressors involving an immediate physiologic threat ('systemic' stressors) are relayed directly to the PVN, probably via brainstem catecholaminergic projections. By contrast, stressors requiring interpretation by higher brain structures ('processive' stressors) appear to be channeled through limbic forebrain circuits. Forebrain limbic sites connect with the PVN via interactions with GABA-containing neurons in the bed nucleus of the stria terminalis, preoptic area and hypothalamus. Thus, final elaboration of processive stress responses is likely to involve modulation of PVN GABAergic tone. The functional and neuroanatomical data obtained suggest that disease processes involving inappropriate stress control involve dysfunction of processive stress pathways.

Trends Neurosci. (1997) 20, 78–84

THE EXPERIENCE OF STRESS is common to all living things. Imposition or perception of environmental or physical change, either negative (that is, threatening) or positive (that is, rewarding), elicits a spectrum of physiologic changes that can be construed as adaptive to the organism. Prominent among these is the release of glucocorticoids by the adrenal glands, which serves both to alert the organism to environmental or physiologic changes and to defend homeostasis.

Unfortunately, inadequate control of glucocorticoid stress responses represents a severe threat to the health and well-being of the organism. Hypersecretion of glucocorticoids can promote the development of physiologic and psychologic dysfunction. For example, inappropriate regulation of stress has been implicated in the pathogenesis of systemic disease (for example, colitis, asthma, hypertension)[1], affective disorders (for example, depression, post-traumatic stress disorder)[2,3] and neurodegenerative disease (for example, Alzheimer's disease)[4]. The development or perpetuation of these disease states might be associated with a temporal prolongation of initially adaptive responses to discrete stressful events.

Glucocorticoid secretion is accomplished by the hypothalamo–pituitary–adrenocortical (HPA) stress axis. The HPA system is in turn controlled by a diverse set of afferents that co-ordinate secretion with the characteristics of provocative stimuli and their physiologic impact. This review summarizes current knowledge of afferent regulation of the HPA axis, and attempts to synthesize this body of data into a working model of central stress control.

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Central co-ordination of glucocorticoid release: role of the paraventricular nucleus

Central control of glucocorticoid secretion is regulated principally by a select population of neurosecretory neurons in the hypothalamic paraventricular nucleus (PVN). Upon stimulation by stress, these neurons secrete a cocktail of adrenocorticotrophic hormone (ACTH) secretagogues, the most important of which are corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP), into the pituitary portal circulation[5]. Subsequent increases in circulating ACTH then drive synthesis and secretion of glucocorticoids by the adrenal cortex. The magnitude of the HPA stress response elicited by these PVN neurons is limited by both neuronal and hormonal mechanisms to maintain glucocorticoid levels within tolerable limits[6].

The PVN appears to be the crucial focus for central regulation of the HPA axis. The PVN is clearly responsible for initiating glucocorticoid secretion, as lesion of this region markedly reduces portal CRH levels and stress-induced ACTH and corticosterone secretion[7]. Stimulation of the HPA system is marked by depletion of CRH- and AVP-containing neurosecretory vesicles in the external lamina of the median eminence, indicative of ACTH secretagogue release[5]. Prolonged stress elicits large increases in the expression of CRH and AVP mRNA in the PVN (Refs 8,9) and enhances co-expression of CRH and AVP in the external lamina of the median eminence[5], suggesting an increased capacity for the action of ACTH secretagogues on the pituitary gland. The animal data are consistent with human post-mortem studies documenting increased expression of CRH mRNA, and CRH and AVP peptide in the PVN of depressed individuals and Alzheimer's disease patients[10,11], suggesting a connection between the stress-induced drive of the PVN and the glucocorticoid dyshomeostasis characteristic of these diseases.

Initiating the stress response

Excitation of the HPA axis is driven by select central stress circuits (Table 1). Notable among these are

  0166 - 2236/97/$17.00 PII: S0166-2236(96)10069-2

TABLE 1. Stress-excitatory circuits

	Brainstem nuclei (A2, C1–C3)	**Forebrain nuclei** (MeA, PMCo, CeA, lateral BST)
Lesion studies: HPA responses affected	Ether Hemorrhage Cytokines Hypoglycemia	Conditioned fear (CeA, BST) Restraint or immobilization (CeA) Photic stimulation (MeA, PMCo) Acoustic stimulation (MeA, PMCo)
Lesion studies: HPA responses unaffected	Footshock Restraint (±)	Ether (CeA, MeA)
IEG expression	Ether Hemorrhage Cytokines Hypoglycemia Footshock Swim Restraint	Swim (MeA, PMCo) Restraint (MeA, PMCo) Footshock (MeA) Cytokines (CeA, BST)
Glucocorticoid-receptor expression	GR	GR (CeA) MR (CeA, MeA, PMCo)
Neuromodulators	NA, A Neuropeptides	GABA (BST, MeA, PMCo>CeA) EAA (all) Neuropeptides (CeA, BST>MeA, PMCo)
Negative feedback	Unknown	Unknown

Table 1 represents a partial summary of available data on structures believed to activate the HPA axis (Refs in text). Lesion studies: HPA responses affected, those stressors whose HPA response is decreased by lesion of indicated structures; Lesion studies: HPA responses unaffected, stressors whose HPA response is not modulated by lesion; IEG expression, stressors inducing IEG expression in indicated structures; Glucocorticoid-receptor expression, receptor subtypes expressed in indicated structures; Neuromodulators, neuroactive substances expressed in indicated structures; Negative feedback, lesion or steroid implants that affect HPA stress responses or response to exogenous glucocorticoids. Abbreviations: A, adrenaline; BST, bed nucleus of the stria terminalis, lateral part; CeA, amygdaloid central nucleus; EAA, excitatory amino acids; GR, glucocorticoid receptor; HPA, hypothalamo–pituitary–adrenocortical; IEG, immediate–early gene; MeA, medial amygdaloid nucleus; MR, mineralocorticoid receptor; NA, noradrenaline; PMCo, posterior cortical amygdaloid nucleus.

brainstem catecholamine-producing pathways, which project directly to CRH-containing neurons of the PVN (Refs 12,13). Catecholaminergic drive appears to promote HPA secretory activity following hemorrhage, hypotension and respiratory distress[14], and might play a role in ACTH responses to immune challenge as well[15]. The excitatory effects of catecholamines on HPA activation appear to be mediated by PVN α-adrenoceptors[14]. Acute stress induces a rapid induction of immediate–early gene expression in brainstem catecholamine neurons[16–18], suggesting a connection between activation of these cell groups and HPA stress responses. Notably, deafferentation of ascending brainstem pathways to PVN inhibits induction of c-*fos* mRNA and protein in hypophysiotrophic neurons following immune challenge, further consistent with an excitatory impact of this cell population on HPA activation[19]. However, deafferentations are completely ineffective in blocking PVN c-*fos* induction by footshock, suggesting use of alternative circuitry by this stressor[19].

Additional HPA-excitatory information might be communicated by way of the amygdala (Table 1). The amygdala is known to prompt behavioral and cardiovascular responses to stress[20]. Damage to the amygdala has been shown to decrease corticosterone and ACTH secretion following leg-break or adrenalectomy[21,22], consistent with an impact of this structure on HPA activation. More-detailed analyses suggest that excitatory effects of the amygdala on HPA function are mediated by the central, medial and cortical amygdaloid nuclei. Stimulation of the medial or cortical amygdaloid nuclei elicits corticosterone secretion[23], consistent with stress-excitatory roles for these regions. This notion is supported indirectly by evidence showing massive c-*fos* induction in these neurons upon restraint or swim stress[17]. Lesion studies corroborate the involvement of the amygdala in HPA excitation; for example, ablation of the medial or central amygdaloid nuclei block HPA responses to acoustic and photic stimulation[24]. Other studies further indicate that lesions of the central nucleus decrease ACTH or corticosterone responses to restraint and fear conditioning[25]. However, medial and central amygdaloid lesions do not block HPA responsiveness to ether[24], providing evidence for stressor-specificity in amygdaloid stress pathways.

The bed nucleus of the stria terminalis (BST) may also convey excitation of the HPA axis. This limbic forebrain structure links regions such as the amygdala and hippocampus with hypothalamic and brainstem regions controlling vital homeostatic functions[26–28]. Specific ablation of lateral divisions of this region decreases expression of CRH mRNA in the PVN (Ref. 29) and attenuates corticosterone secretion induced by conditioned fear[30], whereas stimulation of the lateral BST increases corticosterone secretion[31]. Interestingly, these cell groups are considered by many to be extensions of the central amygdaloid nucleus[32]. This notion is supported somewhat by similar effects of lesions of the central amygdaloid nucleus and lateral BST on the stress axis.

TABLE 2. Stress-inhibitory circuits

	Limbic system nuclei (HPC, VS, PFC, LS)	**BST, hypothalamus, POA nuclei** (medial BST, MPOA, DMH, VMH, ARC, SCN)
Lesion studies: HPA responses affected	Restraint (HPC, PFC, VS, LS) Novelty (HPC, LS)	Restraint (MPOA, ARC, SCN)
Lesion studies: HPA responses unaffected	Hypoxia (HPC) Ether (PFC, VS)	
IEG expression	Swim (PFC, VS, LS>HPC) Restraint (PFC, VS, LS>HPC) Footshock (PFC, LS)	Swim (MPOA, DMH, ARC>BST) Restraint (MPOA, DMH, ARC>BST) Footshock (BST)
Glucocorticoid-receptor expression	GR (HPC, PFC, VS) MR (HPC, LS>PFC,VS)	GR (MPOA, ARC>BST, VMH) MR (MPOA)
Neuromodulators	EAA (PFC, VS, LS>HPC)	GABA (all)
Negative feedback	Restraint (HPC, PFC, VS) (not hypoxia) (HPC) (not ether) (PFC, VS)	Restraint (MPOA, SCN) Circadian (VMH, SCN)

Table 2 represents a partial summary of available data on structures believed to inhibit the HPA axis (Refs in text). Lesion studies: HPA responses affected, stressors whose HPA response is increased by lesion of indicated structures; Lesion studies: HPA responses unaffected, stressors whose HPA response is not modulated by lesion; IEG expression, stressors inducing IEG expression in indicated structures; Glucocorticoid-receptor expression, receptor subtypes expressed in indicated structures; Neuromodulators, neuroactive substances expressed in indicated structures; Negative feedback, lesion or steroid implants that affect HPA stress responses or response to exogenous glucocorticoids. Abbreviations: ARC, arcuate nucleus; BST, bed

Activation of the stress axis may be affected by ascending aminergic input from the locus coeruleus (noradrenaline) and raphé nuclei (5-HT). The role of these regions in HPA regulation is controversial at present. For example, the locus coeruleus is one of the most stress-responsive regions in brain[17,33,34], and has been implicated in regulation of HPA responses to hemorrhage[35]. However, the locus coeruleus has quite limited direct input to the PVN region[12], and thus might exert HPA effects through its dense innervation of central limbic structures. The role of 5-HT in HPA regulation has also been debated; some studies indicate excitatory actions of 5-HT on ACTH and corticosterone release[36], whereas others indicate concentration-dependent facilitatory and inhibitory effects on HPA tone[37,38]. Like the locus coeruleus, direct 5-HT innervation of the PVN is somewhat limited[39], suggesting the potential for indirect actions by way of other stress pathways.

In addition to aminergic and limbic pathways, it is also clear that glutamate-containing and possibly ACh-containing neurons play a role in excitation of the PVN. Injection of glutamate into the region of the PVN activates neurosecretory neurons[40]. Furthermore, parvocellular PVN neurons express NMDA, kainate and AMPA receptors[41], and appear to receive synaptic input from glutamate-containing neurons[42]. The source of glutamate input into the PVN remains to be identified definitively. Acetylcholine is known to enhance CRH release in explant cultures, and to increase ACTH release and expression of CRH mRNA following microinjection into the PVN (Refs 43,44). However, anatomical studies suggest very sparse cholinergic innervation of the PVN proper[45], suggesting that cholinergic actions might be relayed by hypothalamic local circuit neurons.

Limiting the stress response

The importance of maintaining glucocorticoid secretion within tolerable limits requires efficient mechanisms for inhibiting stress-integrative PVN neurons. This process appears to be accomplished by multiple pathways (Table 2). Notably, glucocorticoid injections into the PVN region downregulate CRH mRNA, decrease ACTH secretion and inhibit medial parvocellular PVN neurons[5], suggesting that glucocorticoid negative feedback acts at the PVN neuron itself. The capacity for direct glucocorticoid feedback is supported by evidence of expression of type 2 adrenocorticosteroid receptors (designated as glucocorticoid receptors, or GRs) in hypophysiotrophic PVN neurons[46]. However, feedback at the PVN cannot account for all aspects of HPA inhibition. For example, inhibition of ACTH release occurs in the absence of a negative feedback signal[47]. These data argue for the existence of neuronal inhibitory pathways working in parallel with steroid feedback. Furthermore, total or anterior deafferentations of the PVN increase the expression of CRH and AVP mRNA (Ref. 48), indicating that neuronal inhibitory pathways are required for maintenance of basal HPA tone. Finally, chronic stress-induced increases in glucocorticoid secretion are not sufficient to block upregulation of parvocellular CRH and AVP mRNA expression, despite the presence of glucocorticoid receptors in these neurons[8,9,49].

Neuronally mediated inhibition of the HPA axis might emanate from several sources. Perhaps the most intensely studied of these has been the hippocampus.

The hippocampus displays the highest levels of glucocorticoid binding, and GR and mineralocorticoid receptor [high-affinity (type 1) adrenocorticosteroid receptor] mRNA of any brain structure, suggesting a high degree of glucocorticoid receptivity[50,51]. An inhibitory role for the hippocampus in HPA regulation is supported by lesion studies, which indicate that hippocampal damage potentiates stress-induced glucocorticoid secretion in rat and primate, and increases the expression of CRH and AVP mRNA in parvocellular PVN neurons[51–54]. Conversely, stimulation of the hippocampal formation results in decreased HPA activity in both rat and human[51]. These studies are consistent with an inhibitory role of the hippocampus on the HPA axis. At present, effects of the hippocampus on glucocorticoid negative feedback are controversial; some studies suggest that hippocampal lesion attenuates that ability of exogenous glucocorticoids to inhibit stress responses, whereas others do not[51,55].

Other structures in the limbic system appear to convey some degree of inhibition to the PVN. Damage to either the prefrontal cortex or lateral septum results in enhanced HPA responsiveness to acute stress[56,57]. Implants of glucocorticoids into the prefrontal cortex block restraint-induced ACTH secretion[57], implicating this region in glucocorticoid negative feedback processes as well. In addition, both the prefrontal cortex and the septum exhibit a massive induction of immediate–early gene expression following acute stress[17], consistent with a role in integration of stressful information.

It is important to note that limbic stress-inhibitory circuits operate in a stressor-specific manner. For example, lesions in the prefrontal cortex increase restraint-induced ACTH and corticosterone release, but do not affect responses to stress induced by ether[57]. Likewise, hippocampal damage increases corticosterone responses to restraint[58], but are ineffective in modulating ACTH and corticosterone responses to hypoxia[55].

The PVN neuron receives direct inhibitory input from local hypothalamic circuits. Lesion studies indicate that several local PVN-projecting cell groups (including the BST, preoptic area and hypothalamus) have the capacity to inhibit HPA activation. Ablations of the arcuate nucleus, medial preoptic area, ventromedial nucleus or suprachiasmatic nucleus increase basal ACTH or corticosterone secretion, and the magnitude and duration of HPA stress responses[59–62]. Lesions of the medial BST increase the expression of CRH mRNA in the PVN (Ref. 29), whereas stimulation of this region decreases corticosterone release[31]. Importantly, all of these regions contain substantial populations of GABA-containing neurons[63]. GABA is known to inhibit the release of ACTH and corticosterone *in vivo*[64] and reduce CRH release from hypothalamic explants[65,66], suggesting that GABA interacts directly with hypophysiotrophic PVN neurons. Direct GABA actions on the HPA axis are further supported by the presence of GABA-immunoreactive terminals on parvocellular PVN neurons[42], and by evidence localizing $GABA_A$ receptors to neurons of the medial parvocellular PVN (Ref. 67).

The potential for glucocorticoids to exert negative feedback action by way of hypothalamic cell groups is highlighted by rich expression of GR protein and mRNA throughout the preoptic–hypothalamic continuum[50]. In line with this notion, ventromedial hypothalamic lesions decrease the ability of low doses of corticosterone to inhibit baseline ACTH release[62]. Furthermore, recent data indicate that implants of corticosterone into the medial preoptic area inhibit HPA responses to restraint and reduce AVP content in the median eminence[68].

Hypothesis: 'processive' vs 'systemic' stress pathways

The literature suggests that the stress-regulatory circuit activated by a particular stressor is crucially dependent on stimulus attributes (see Tables 1 and 2). In general, limbic stress pathways are most sensitive to stressors involving higher-order sensory processing. For example, HPA responses to restraint, fear conditioning or exposure to a novel environment are affected by lesions of the prefrontal cortex, hippocampus or amygdala. These stressors have common features: (1) all require assembly and processing of signals from multiple sensory modalities prior to initiation of a stress response; and (2) none of the listed stressors involve an immediate threat to physiologic homeostasis, but rather constitute stimuli that become stressful (or unstressful) only by comparison with previous experience. By contrast, HPA responses to physiologic threats, such as ether or hypoxia, are not affected by lesions of the limbic system. These stressors also have common properties: (1) both can be relayed directly to the PVN by visceral efferent pathways; and (2) both elicit a respiratory distress constituting a direct threat to survival. In these situations, a case can be made for rapid relay of an excitatory signal to the PVN by way of brainstem circuitry, bypassing the need for cognitive processing.

The marked distinction between limbic-sensitive and limbic-insensitive stressors leads us to postulate the existence of two generalized stress pathways. The former, limbic-sensitive stressors are 'processive', in that they require a sequential stimulus assembly to obtain physiologic meaning. In this case, multimodal stimuli are assembled at the cortical level and the trace diverted to multiple structures. It is likely that the impact of complex stimuli is channeled to the HPA axis as one part of the integrated limbic response to novel or threatening information. Limbic circuits are then capable of augmenting or diminishing the resultant HPA response, depending on prior experience or ongoing level of activation. Limbic-insensitive stressors, including the respiratory stressors noted above and perhaps cardiovascular and immune stimuli as well, represent 'systemic' stressors (see Sawchenko and colleagues[18]). These stressors are of immediate survival value and do not require interpretation by higher-order brain structures; rather, they gain access to the PVN by a relatively direct pathway (Fig. 1). For example, anatomical evidence indicates that information on blood oxygenation is relayed from sensory elements in the carotid body or carotid sinus to the PVN by way of a single synapse with (catecholamine-containing) neurons in the nucleus of the solitary tract or ventrolateral medulla[69,70]. The directness of this pathway obviates the need for processing by higher brain structures, and is likely to reflect the overwhelming importance of regaining cardiovascular or respiratory homeostasis.

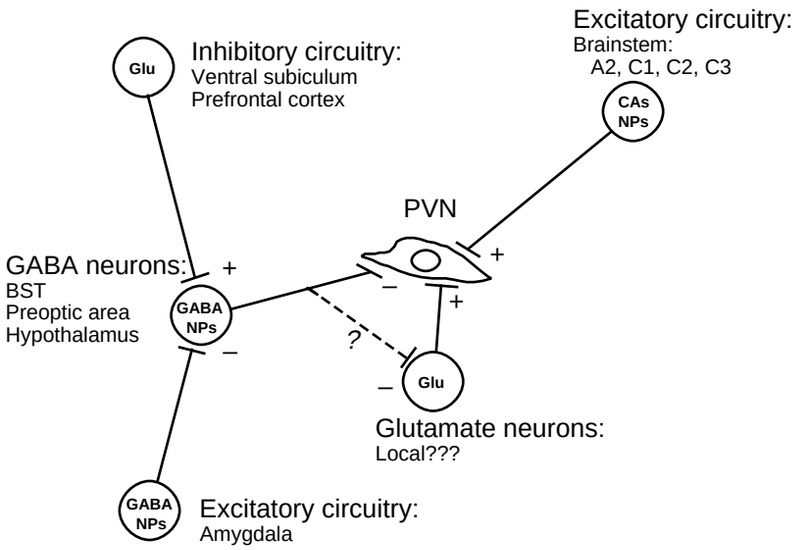

Fig. 1. ***Schematic representation of central stress circuitry.*** *According to this scenario, stimuli integrated by way of the 'processive' stress pathway project to GABA-containing neurons in the bed nucleus of the stria terminalis (BST), preoptic area and hypothalamus. Inhibitory circuits present excitatory output to GABA-containing neurons, resulting in an increase in inhibition at the paraventricular nucleus (PVN). Excitatory circuits present inhibitory input to GABA-containing neurons, attenuating inhibition at the PVN. Information following 'systemic' pathways, by contrast, project directly to the PVN and activate the hypothalamic–pituitary–adrenocortical (HPA) axis. The role of glutamate is ill-defined at present; glutamate appears to activate PVN neurons, indicative of an excitatory role. However, the source of glutamatergic input is unknown, as are potential interactions between glutamate and GABA neurons. Abbreviations: CAs, catecholamines; Glu, glutamate; NPs, neuropeptides.*

The distinction between processive and systemic stressors does not assume that all stressors within the two classes use identical circuitry. Different types of processive stressors might use quite distinct sensory and associative pathways prior to interacting with structures from the limbic system. Indeed, restraint stress, which involves restricted movement, shows different patterns of central c-*fos* mRNA induction than swim stress, which encourages movement[17]. It then follows that specific stressors should elicit characteristic patterns of limbic activation. Thus, the eventual impact of an individual processive stressor on the PVN reflects the distinct set of limbic relays it employs.

One of the key characteristics of processive stress integration is the apparent need for an intervening synapse between limbic sites (prefrontal cortex, hippocampus and amygdala) and the PVN. Anatomical studies suggest that stress-regulatory limbic sites lack substantial direct input to hypophysiotrophic PVN neurons (see Ref. 71), implying that physiologic actions on the HPA are indirect. For the hippocampus and amygdala, interactions are likely to occur among contingents of PVN-projecting cells in the preoptic area, hypothalamus and medial region of the BST. We have performed anatomical studies comparing the distribution of limbic efferents on neurons projecting to the PVN (Refs 26,72; Fig. 2). Our data indicate that labeled efferents from the ventral subiculum contact PVN-projecting neurons in the BST, medial preoptic area, anterior hypothalamus, subparaventricular region and dorsomedial hypothalamus, suggesting that monosynaptic hippocampal–PVN relays involve neurons in these regions[26]. The vast majority of these PVN-projecting neurons contain GABA (Ref. 26). It is notable that the hippocampus is known to increase activity following exposure to novel or stressful stimuli, perhaps as part of its role in central memory processing and stimulus–expectancy comparisons (see Ref. 74). Interaction of hippocampal efferents with GABA-containing neurons from BST, preoptic area or hypothalamus suggests a capacity for excitatory outflow from the hippocampus to achieve a net inhibitory action on the PVN (Fig. 1).

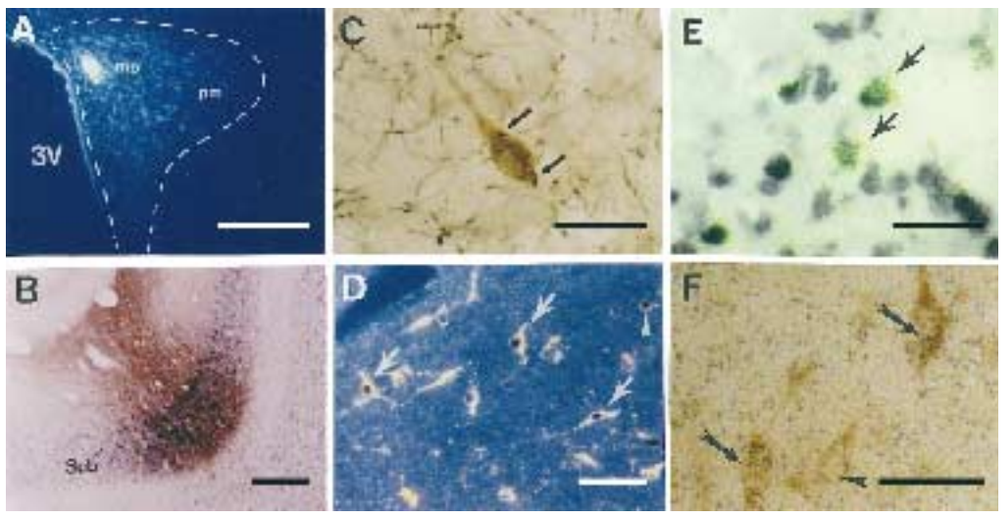

Fig. 2. ***Photomicrographs illustrating anatomical interactions among forebrain and limbic structures and the hypothalamic paraventricular nucleus (PVN).*** **(A** ***and*** **B)** *Deposition of the retrograde neuronal tracer Fluorogold in the PVN is illustrated* (A), *as well as an injection of the anterograde neuronal tracer PHAL located within the ventral subiculum* (B). **(C)** *The results of a double-immunolabeling experiment following tracer injections similar to those in* A *and* B*; a Fluorogold immunolabeled neuron (brown) within the bed nucleus of the stria terminalis (BST) is apposed by PHAL-labeled fibers and terminals (black), suggestive of a subiculum–BST–PVN pathway.* **(D)** *Darkfield photomicrograph illustrates the results of a double-immunolabeling study performed in animals subjected to acute swim stress; the protein product of the immediate–early gene c-*fos, *used as a neuronal activation marker, has been detected in combination with Fluorogold following deposition of the tracer in the PVN. Solid white arrows depict Fluorogold-labeled neurons located in the preoptic area, which are immunopositive for the nuclear c-fos protein, indicative of PVN afferent cells that are stress-responsive.* **(E)** *A dual* in situ *hybridization histochemical procedure has been applied for simultaneous detection of mRNA transcripts encoding glutamic acid decarboxylase (GAD; purple cells), the GABA-synthesizing enzyme, and c-fos (green grains) in animals subjected to acute restraint stress. Arrows indicate GAD–fos double-labeled neurons located in the anterior hypothalamic area.* **(F)** *Fluorogold immunocytochemistry is combined with hybridization histochemistry for GAD in an animal in which the retrograde tracer was delivered to the PVN; arrows depict BST neurons immunolabeled for Fluorogold (brown) that express GAD (indicated by collections of black grains). Collectively, the combined applications of these techniques have provided anatomical support for hippocampal projections to forebrain neurons that are, in turn, capable of inhibiting cells within the PVN in the context of an acute stress response. Scale bars, 250 μm* (A), *500 μm* (B), *50 μm* (C–E), *25 μm* (F). *Abbreviations: 3V, third ventricle; mp, medial parvocellular PVN; pm, posterior magnocellular PVN; Sub, subiculum.* Portions of this figure are reprinted, with permission, from Refs 26,73.

Efferents from medial and posterior cortical amygdala innervate PVN-projecting cell groups in the BST, preoptic area and hypothalamus[72], suggesting that these regions also interact with GABA-containing PVN projections. Unlike the hippocampus, both the medial and cortical amygdala possess substantial populations of presumptive GABA and neuropeptide-containing projection neurons[75,76], indicating the potential for these nuclei to disinhibit PVN-projecting GABA neurons in BST, preoptic area or hypothalamus, and thereby increase HPA activation (Fig. 1).

Many details of the local hypothalamic neurocircuitry linking

limbic forebrain structures to the PVN remain to be resolved. For example, it is unclear whether local glutamate-containing relay neurons play a major role in PVN activation. Similarly, the source and regulatory actions of neuropeptide-containing afferents are obscure, at present. However, it is important to note the intersection of limbic circuitry with hypothalamic regions capable of communicating homeostatic information to the PVN. Relay through these regions allows limbic information to be processed with respect to ongoing physiological status. Through such pathways, the salience of stressful stimuli stands to be corrected for caloric requirements, fluid balance, thermoregulatory status and endogenous rhythmicity prior to interacting with PVN neurons. The potential for hypothalamic modulation of the interaction between the limbic system and PVN is evident from the impact of hypothalamic lesion on HPA activation, and from the ability of stress to activate PVN-projecting hypothalamic cell groups (as noted above). Furthermore, the mainly GABAergic phenotype of this region[63,77] is reflected in the generally inhibitory impact of these structures on HPA secretory activity. Based on the available data, we postulate that the BST, preoptic and hypothalamic cell groups integrate limbic input with homeostatic information prior to final elaboration of the stress response. These circuits might then modulate PVN output through enhancement or withdrawal of GABAergic tone (Fig. 1).

Brain stress regulation in disease

Neuronally mediated control of the hypothalamic PVN (and subsequent glucocorticoid secretion) is clearly crucial for maintenance of health and well-being under basal and 'stressful' conditions. The data reviewed above add important insight to the understanding of human stress-related HPA pathology. For example, alterations in neuroendocrine control induced by life stresses are likely to be associated with limbic dysfunction, involving regions such as the hippocampal formation, medial prefrontal cortex or the amygdaloid body. Neuroendocrine changes probably reflect only one aspect of this dysfunction. While a connection between the limbic system and human stress pathology has yet to be firmly established, it is of considerable interest to note that imaging studies link changes in the activity or volume of the amygdala, prefrontal cortex and hippocampus with major depression[78,79], a disease marked by hyperactivity of the HPA stress axis. In addition, the neurocircuitry data suggest that GABA-containing pathways might comprise a key component of the abnormalities in the HPA axis seen in human stress pathology. This point has yet to be addressed definitively; however, it is clear that GABA modulates stress-induced glucocorticoid secretion in both rat and human[64,80], and has been identified as a mitigating factor in major depressive illness[81].

Future investigations aimed at pinpointing neural pathways controlling the stress axis might benefit from the distinctions between systemic and processive stress circuits outlined above. Clearly, control of the secretion of stress hormones is distributed throughout multiple brain regions capable of interpreting the significance of evocative stimuli with respect to prior experience, level of arousal and systemic homeostasis. The formidable task of defining key elements of HPA control requires further interdisciplinary approaches aimed at understanding stress-responsive brain pathways in terms of interaction between local and distant cell networks. Future analyses of central stress integration need also recognize the constant interplay between activational and inhibitory circuitries, and the multiple levels at which steroids themselves might intervene in regulating optimal glucocorticoid output.

Selected references

1 **McEwen, B.S. and Stellar, E.** (1993) *Arch. Intern. Med.* 153, 2093–2101
2 **Kathol, R.G., Jaeckle, R.S. and Lopez, J.F.** (1989) *Am. J. Psychiatry* 146, 311–317
3 **Charney, D. *et al.*** (1993) *Arch. Gen. Psychiatry* 50, 295–305
4 **Landfield, P.W. and Eldridge, J.C.** (1991) *Acta Endocrinol.* 125, 54–64
5 **Whitnall, M.H.** (1993) *Prog. Neurobiol.* 40, 573–629
6 **Keller-Wood, M. and Dallman, M.F.** (1984) *Endocr. Rev.* 5, 1–24
7 **Makara, G.B.** (1992) *Ciba Found. Symp.* 168, 43–51
8 **Sawchenko, P.E., Arias, C.A. and Mortrud, M.T.** (1993) *J. Neuroendocrinol.* 5, 341–348
9 **Herman, J.P., Adams, D. and Prewitt, C.M.** (1995) *Neuroendocrinology* 61, 180–190
10 **Raadsheer, F.C. *et al.*** (1995) *Am. J. Psychiatry* 152, 1372–1376
11 **Raadsheer, F.C. *et al.*** (1994) *Neuroendocrinology* 60, 436–444
12 **Cunningham, E.T., Jr and Sawchenko, P.E.** (1988) *J. Comp. Neurol.* 274, 60–76
13 **Cunningham, E.T., Jr, Bohn, M.C. and Sawchenko, P.E.** (1990) *J. Comp. Neurol.* 292, 651–667
14 **Plotsky, P.M., Cunningham, E.T., Jr and Widmaier, E.P.** (1989) *Endocr. Rev.* 10, 437–458
15 **Ericsson, A., Kovacs, K.J. and Sawchenko, P.E.** (1994) *J. Neurosci.* 14, 897–913
16 **Chan, R.K.W. *et al.*** (1993) *J. Neurosci.* 13, 5125–5138
17 **Cullinan, W.E. *et al.*** (1995) *Neuroscience* 64, 477–505
18 **Sawchenko, P.E. *et al.*** (1996) *Prog. Brain Res.* 107, 201–225
19 **Li, H-Y., Ericsson, A. and Sawchenko, P.E.** (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 2359–2364
20 **Davis, M.** (1992) *Annu. Rev. Neurosci.* 15, 353–375
21 **Allen, J.P. and Allen, C.F.** (1974) *Neuroendocrinology* 15, 220–230
22 **Allen, J.P. and Allen, C.F.** (1975) *Neuroendocrinology* 19, 115–125
23 **Dunn, J.D. and Whitener, J.** (1986) *Neuroendocrinology* 42, 211–217
24 **Feldman, S. *et al.*** (1994) *Brain Res.* 658, 21–26
25 **Van de Kar, L.D. *et al.*** (1991) *Neuroendocrinology* 54, 89–95
26 **Cullinan, W.E., Herman, J.P. and Watson, S.J.** (1993) *J. Comp. Neurol.* 332, 1–20
27 **Moga, M.M., Saper, C.B. and Gray, T.S.** (1989) *J. Comp. Neurol.* 283, 315–332
28 **Weller, K.L. and Smith, D.A.** (1982) *Brain Res.* 232, 255–270
29 **Herman, J.P., Cullinan, W.E. and Watson, S.J.** (1994) *J. Neuroendocrinol.* 6, 433–442
30 **Gray, T.S. *et al.*** (1993) *Neuroendocrinology* 57, 517–524
31 **Dunn, J.D.** (1987) *Brain Res.* 407, 327–331
32 **de Olmos, J., Alheid, G.F. and Beltramino, C.A.** (1985) in *The Rat Nervous System* (Paxinos, G., ed.), pp. 223–307, Academic Press
33 **Abercrombie, E.D. and Jacobs, B.L.** (1987) *J. Neurosci.* 7, 2837–2843
34 **Smith, M.A. *et al.*** (1991) *Brain Res.* 544, 26–32
35 **Gann, D.S. *et al.*** (1977) *Ann. New York Acad. Sci.* 297, 477–496
36 **Feldman, S., Conforti, N. and Melamed, E.** (1987) *Brain Res. Bull.* 18, 165–168
37 **Korte, S.M. *et al.*** (1991) *Eur. J. Pharmacol.* 197, 225–228
38 **Welch, J.E. *et al.*** (1993) *Neuroendocrinology* 57, 272–281
39 **Sawchenko, P.E. *et al.*** (1983) *Brain Res.* 277, 355–360
40 **Tasker, J.G. and Dudek, F.E.** (1993) *J. Physiol.* 469, 179–192
41 **van den Pol, A. *et al.*** (1994) *J. Comp. Neurol.* 343, 428–444
42 **Decavel, C. and van den Pol, A.N.** (1992) *J. Comp. Neurol.* 316, 104–116
43 **Ohmori, N. *et al.*** (1995) *Endocrinology* 136, 4858–4863
44 **Hillhouse, E.W. and Milton, N.G.N.** (1989) *J. Endocrinol.* 122, 713–718
45 **Ruggiero, D.A. *et al.*** (1990) *J. Comp. Neurol.* 292, 1–53
46 **Uht, R.M. *et al.*** (1988) *J. Neurosci. Res.* 19, 405–411
47 **Jacobson, L. *et al.*** (1988) *Endocrinology* 122, 1343–1348
48 **Herman, J.P., Wiegand, S.J. and Watson, S.J.** (1990) *Endocrinology* 127, 2408–2417
49 **Makino, S., Smith, M.A. and Gold, P.W.** (1995) *Endocrinology* 136, 3299–3309
50 **Herman, J.P.** (1993) *Cell. Mol. Neurobiol.* 13, 349–372
51 **Jacobson, L. and Sapolsky, R.M.** (1991) *Endocr. Rev.* 12, 118–134
52 **Herman, J.P. *et al.*** (1989) *J. Neurosci.* 9, 3072–3082
53 **Herman, J.P. *et al.*** (1995) *J. Neuroendocrinol.* 7, 475–482
54 **Sapolsky, R.M., Zola-Morgan, S. and Squire, L.R.** (1991) *J. Neurosci.* 11, 3695–3704

55 Bradbury, M.J., Strack, A.M. and Dallman, M.F. (1993) *Neuroendocrinology* 58, 396–407
56 Dobrakovova, M., Kvetnansky, R. and Torda, T. (1982) *Physiol. Behav.* 29, 41–45
57 Diorio, D., Viau, V. and Meaney, M.J. (1993) *J. Neurosci.* 13, 3839–3847
58 Sapolsky, R.M., Krey, L.C. and McEwen, B.S. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 6174–6177
59 Viau, V. and Meaney, M.J. (1991) *Endocrinology* 129, 2503–2511
60 Buijs, R.M. *et al.* (1993) *Am. J. Physiol.* 264, R1186–R1192
61 Larsen, P.J. *et al.* (1994) *J. Endocrinol.* 141, 497–503
62 Suemaru, S. *et al.* (1995) *Neuroendocrinology* 61, 453–463
63 Okamura, H. *et al.* (1990) *Neuroscience* 39, 675–699
64 Makara, G.B. and Stark, E. (1974) *Neuroendocrinol.* 16, 178–190
65 Hillhouse, E.W. and Milton, N.G.N. (1989) *J. Endocrinol.* 122, 719–723
66 Calogero, A.E. *et al.* (1988) *Brain Res.* 463, 28–36
67 Fritschy, J.M. and Mohler, H. (1995) *J. Comp. Neurol.* 359, 154–194
68 Viau, V. and Meaney, M.J. (1996) *J. Neurosci.* 16, 1866–1876
69 Kalia, M. and Welles, R.V. (1980) *Brain Res.* 188, 23–32
70 Swanson, L.W. and Sawchenko, P.E. (1983) *Annu. Rev. Neurosci.* 6, 269–324
71 Swanson, L.W. (1987) in *Handbook of Chemical Neuroanatomy* (Björklund, A., Hökfelt, T. and Swanson, L.W., eds), pp. 1–124, Elsevier
72 Prewitt, C.M. and Herman, J.P. (1995) *Soc. Neurosci. Abstr.* 21, 1389
73 Cullinan, W.E., Helmrich, D.L. and Watson, S.J. (1996) *J. Comp. Neurol.* 368, 88–99
74 Gray, J.A. and Rawlins, J.N.P. (1986) in *The Hippocampus* (Isaacson, R.L. and Pribram, K.H., eds), pp. 159–202, Plenum Press
75 Pitkanen, A. and Amaral, D.G. (1994) *J. Neurosci.* 14, 2200–2224
76 Mugnaini, E. and Oertel, W.H. (1985) in *GABA and Neuropeptides in the CNS* (Vol. 1) (Björklund, A. and Hökfelt, T., eds), pp. 436–622, Elsevier
77 Decavel, C. and van den Pol, A.N. (1990) *J. Comp. Neurol.* 302, 1019–1037
78 Sapolsky, R.M. (1996) *Science* 273, 749–750
79 Drevets, W.C. *et al.* (1992) *J. Neurosci.* 12, 3628–3641
80 Jones, M.T. *et al.* (1984) *Psychoneuroendocrinology* 9, 107–123
81 Petty, F. (1995) *J. Affect. Disord.* 34, 275–281

GAP-43: an intrinsic determinant of neuronal development and plasticity

Larry I. Benowitz and Aryeh Routtenberg

Several lines of investigation have helped clarify the role of GAP-43 (F1, B-50 or neuromodulin) in regulating the growth state of axon terminals. In transgenic mice, overexpression of GAP-43 leads to the spontaneous formation of new synapses and enhanced sprouting after injury. Null mutation of the *GAP-43* gene disrupts axonal pathfinding and is generally lethal shortly after birth. Manipulations of *GAP-43* expression likewise have profound effects on neurite outgrowth for cells in culture. GAP-43 appears to be involved in transducing intra- and extracellular signals to regulate cytoskeletal organization in the nerve ending. Phosphorylation by protein kinase C is particularly significant in this regard, and is linked with both nerve-terminal sprouting and long-term potentiation. In the brains of humans and other primates, high levels of GAP-43 persist in neocortical association areas and in the limbic system throughout life, where the protein might play an important role in mediating experience-dependent plasticity.

Trends Neurosci. (1997) 20, 84–91

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ALTHOUGH MOST MAJOR EVENTS in brain development – neuronal proliferation and migration, axonal outgrowth, target recognition, and programmed cell death – are completed by the early postnatal period, the detailed sculpting of synaptic connections continues for an extended period, perhaps even throughout life. Cajal[1] proposed that:

> 'The extension, growth, and multiplication of neural processes do not stop at the time of birth; they continue beyond that; and nothing is more striking than the difference that exists between the newborn and the adult from the point of view of the length and number of second and third order neuronal processes. Usage is without doubt a basic feature of these modifications…
>
> The expansion of newly formed cellular processes do not advance haphazardly; they are determined by the dominant patterns of neural activity, or by repeated intercellular associations that result from voluntary (mental) associations. It is thought that the expansion of these new associations are accompanied by active growth…'

The diversity of experimental contexts in which GAP-43 was discovered offers the first clue that this protein might provide a link between events that occur during neural development and activity-dependent changes in the mature brain. In studies beginning in the mid-1970s, F1 and B-50 were identified as synaptic phosphoproteins regulated by Ca^{2+} and various peptides[2,3]. This protein was subsequently shown to be a major presynaptic substrate of protein kinase C (PKC)[4,5] and to undergo a persistent change in phosphorylation during long-term potentiation[6]. In an entirely different setting, two groups in the early 1980s described an acidic membrane protein whose expression increased by two orders of magnitude during the course

  0166 - 2236/97/$17.00 PII: S0166-2236(96)10072-2