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Local circuit regulation of paraventricular nucleus stress integration Glutamate–GABA connections

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Abstract

Limbic neurocircuits play a central role in regulation of the hypothalamic–pituitary–adrenocortical (HPA) axis. Limbic influences on adrenocortical hormone secretion are mediated by transynaptic activation or inhibition of hypophysiotrophic neurons in the medial parvocellular paraventricular nucleus (PVN). Projections from the ventral subiculum, prefrontal cortex, medial amygdala, lateral septum, paraventricular thalamus and suprachiasmatic nucleus (SN) terminate in the immediate surround of the PVN, an area heavily populated by GABAergic interneurons. As such, these regions are positioned to modulate paraventricular output via excitation or inhibition of interneuronal projections into the PVN. In addition, the same limbic and diencephalic regions have projections to local PVN-projecting hypothalamic and basal telencephalic nuclei, including the dorsomedial and medial preoptic nuclei and the bed nucleus of the stria terminalis. These regions are involved in both inhibitory and excitatory regulation of the stress axis, indicating that they contain heterogeneous neuronal populations whose relative impact on the PVN is determined by the nature of afferent stimuli. Thus, limbic modulation of the pituitary–adrenocortical system appears to be a multisynaptic process integrated at the level of local PVN-projecting neurocircuits. Local circuits are likely the primary integrators of anticipatory stress responses, and may indeed be the focus of HPA dysfunction seen with aging or affective disease.

Keywords: Hippocampus; Amygdala; Hypothalamus; GABA; Glutamate; Hypothalamic–pituitary–adrenocortical axis; Glucocorticoids

1. Introduction

The hypothalamic–pituitary–adrenocortical (HPA) axis is critical for adaptation and survival of all vertebrate species. Glucocorticoids, the endpoint of HPA activation, promote adaptation by diverting resources to cope with physiological challenge. Glucocorticoid mobilization serves as a double-edged sword. In the short run, glucocorticoids facilitate physiological adaptation; however, if secretion is prolonged, the same processes promote a dyshomeostasis marked by immune deficiency, neuroendocrine/autonomic disturbances and tissue atrophy (McEwen and Stellar, 1993). Thus, it is essential to maintain tight control of HPA activity, in terms of both initiation and cessation of stress responses.

Activation of the HPA axis is controlled by a discrete set of neurosecretory neurons localized in the medial parvocellular subdivision of the hypothalamic paraventricular nucleus (PVN) (Antoni, 1986; Whitnall, 1993). These neurons integrate excitatory and inhibitory signals into appropriate secretion of ACTH secretagogues [corticotropin-releasing factor (CRH) and arginine vasopressin (AVP)], and thus mediate the magnitude and duration of the stress response.

Anatomical analyses of the connectivity of the parvocellular PVN indicate interactions with neuronal systems subserving homeostasis, memory and emotionality (see (Berk and Finkelstein, 1981; Cunningham and Sawchenko, 1988; Robinson et al., 1988; Sawchenko and Swanson, 1983; Silverman et al., 1981; ter Horst and Luiten, 1987; ter Horst et al., 1989)). Inputs concerned with homeostasis emanate predominantly from a circum-

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Table 1
Limbic projections to peri-PVN neurons[a]

Region	Neurotransmitter	Innervation
Ventral subiculum	Glutamate	dense
Medial prefrontal (infralimbic) cortex	Glutamate	moderate
Lateral septum (ventral)	GABA	moderate–dense
Medial amygdaloid nucleus	GABA, neuropeptides	moderate
Paraventricular thalamic nucleus		moderate
Suprachiasmatic nucleus	GABA, neuropeptides	dense
Ventromedial hypothalamic nucleus	GABA	dense

[a] The peri-PVN region includes the subparaventricular zone, scattered cell populations immediately dorsal and lateral to the PVN proper, medial perifornical nucleus and dorsomedial anterior hypothalamic area.

scribed set of medial parvocellular PVN-projecting afferents resident in the brainstem, hypothalamus and basal forebrain. In contrast, limbic mnemonic and emotive circuits access the PVN indirectly; in fact, many descending limbic efferents contact hypothalamic and forebrain neurons that in turn project to the parvocellular PVN zone (Cullinan et al., 1993; Prewitt and Herman, 1998) (Table 1). Thus, neurocircuitry that drives or inhibits the HPA response to cognitive stimuli requires integration proximal to the PVN.

Recent work suggests that a substantial proportion of PVN excitation and inhibition is in fact gated by local circuit neurons in the immediate vicinity of the PVN. These include neurons in the peri-PVN region, as well as a constellation of local intrahypothalamic and proximal basal forebrain connections. In the present review, we present evidence in support of a role for local circuits in limbic–PVN regulation of the HPA axis.

2. Limbic afferents to local PVN-projecting circuitry

The area immediately surrounding the PVN is richly innervated by a number of limbic structures implicated in HPA regulation, including the ventral subiculum, prefrontal cortex, lateral septum, medial amygdala, paraventricular thalamic nucleus and suprachiasmatic nucleus (SCN) (Canteras et al., 1995; Moga et al., 1995; Ongur et al., 1998; Risold and Swanson, 1997; Watts et al., 1987) (Table 1). Anterograde tracing studies indicate that these regions share the property of densely innervating the PVN surround, prominently including the subparaventricular zone, while almost completely avoiding the medial parvocellular PVN. Notably, medial parvocellular PVN neurons show very limited dendritic trees largely contained within the PVN itself (Rho and Swanson, 1989); thus, terminal boutons of limbic afferents to the peri-PVN region (defined as the subparaventricular zone and the region immediately adjacent the lateral and dorsal limits of the PVN proper) do not appear to contact the parvocellular neurons, but rather act through local interneurons.

A substantial proportion of local neurons contacted by descending limbic projections are likely to be GABAergic in phenotype. Previous studies indicate that the vast majority of PVN-projecting neurons in the hypothalamus and bed nucleus of the stria terminalis are glutamic acid decarboxylase (GAD)-positive (Bowers et al., 1998; Cullinan et al., 1993, 1996). Indeed, the immediate surround of the PVN is particularly rich in GABAergic neurons that project into the PVN (Cullinan et al., 1993; Roland and Sawchenko, 1993). These cell populations are stress-responsive, showing up-regulation of the GABA synthesizing enzyme GAD (Bowers et al., 1998) as well as *c-Fos* induction following stress exposure (Cullinan et al., 1996). As such, these neurons are putative targets for descending information relevant to HPA function.

Evidence also exists for local glutamate excitation of PVN neuronal activity (Boudaba et al., 1997). The source of this innervation is unclear, given the lack of a definitive marker for glutamatergic neurons. The extent of interaction between local glutamate neurons and limbic afferents is unknown.

Previous studies support the existence of limbic system—PVN relays as nodal points in HPA integration. Specific limbic regions involved in regulation of PVN function include the ventral subiculum, prefrontal cortex, medial amygdala, lateral septum and paraventricular thalamus.

2.1. Ventral subiculum

The ventral subiculum densely innervates the peri-PVN region, avoiding the PVN proper (Cullinan et al., 1993) (Fig. 1). Notably, ventral subiculum fibers form a dense plexus in the subparaventricular zone (Canteras and Swanson, 1992; Cullinan et al., 1993), an area heavily innervated by other limbic sites as well (see below). In addition to direct connections with the peri-PVN area, the ventral subiculum richly innervates the ventrolateral region of the dorsomedial nucleus, medial preoptic area and anterior medial, posterior intermediate and ventrolateral subnuclei of the bed nucleus of the stria terminalis, all of which in turn send projections to the medial parvocellular PVN.

Previous studies indicate a prominent involvement of the ventral subiculum in integration of psychogenic stressors. For example, damage to the ventral subiculum or its major outflow pathway (lateral fimbria–fornix) up-regulates CRH mRNA expression in the medial parvocellular PVN and enhances corticosterone secretion following restraint or open-field stress, but not ether or hypoxia (Bradbury et al., 1993; Herman et al., 1992, 1995, 1998). The data suggest a role for this structure in integration of HPA responses to stressors that employ hippocampal neurocircuitry, but not those mediated largely through ascending baroreceptor/chemoreceptor inputs.

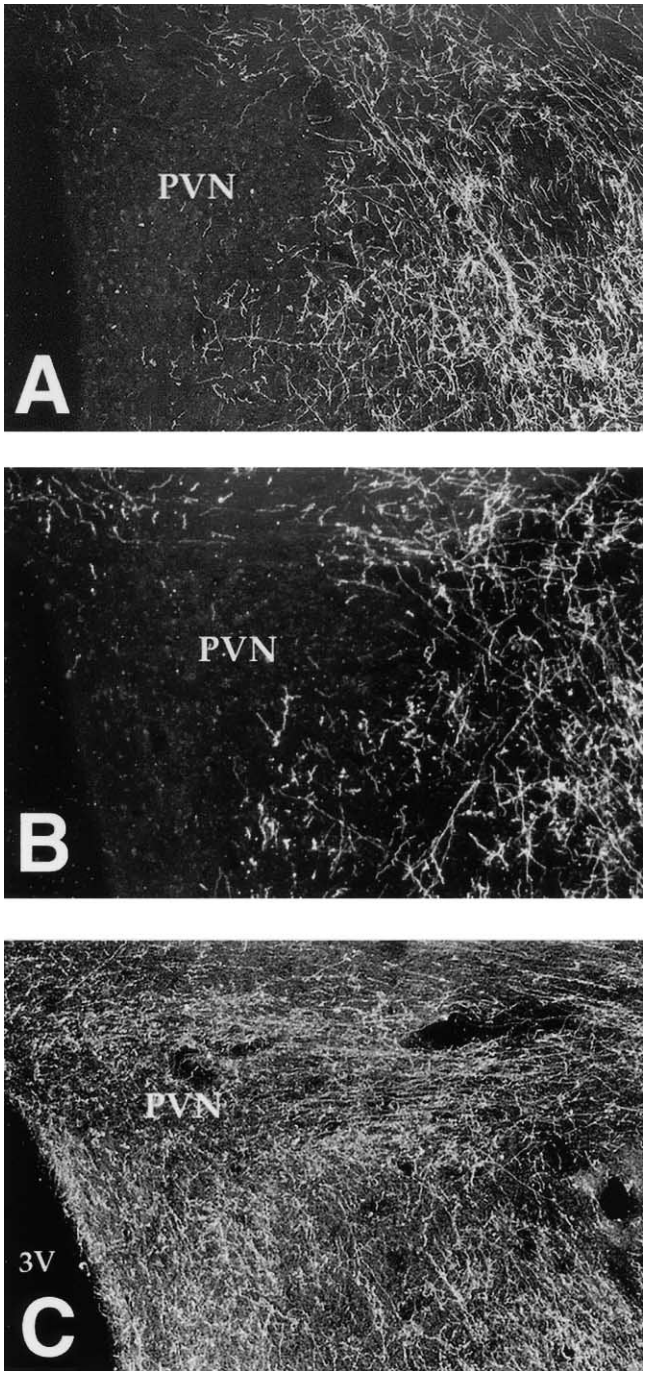


Fig. 1. Darkfield photomicrographs illustrate the pattern of innervation of the PVN and surrounding region following injections of the anterograde tracer PHA-L into the ventral subiculum (A), lateral septal nucleus (B) and the posterior intermediate/anterior medial subnucleus of the bed nucleus of the stria terminalis (C). Note that both subicular and septal projections show terminal boutons in the peri-PVN region, but avoid the PVN proper. In contrast, projections from the bed nucleus of the stria terminalis densely innervate the medial parvocellular PVN.

2.2. Prefrontal cortex

Like the ventral subiculum, the prefrontal cortex has descending projections to the region of the PVN, but not to the PVN itself (Ongur et al., 1998). The anatomical data suggest that this input is less robust than that seen for the ventral subiculum. Interactions between the prefrontal cortex and PVN may also be mediated through projections to the lateral hypothalamus and perifornical region, as well as more limited input to the medial preoptic area, anteroventral periventricular area, dorsomedial hypothalamic nucleus and zona incerta (Hurley et al., 1991; Ongur et al., 1998; Sesack et al., 1989).

Damage to the anterior cingulate/medial prefrontal cortex also precipitates hypersecretion of corticosterone in a stressor-specific manner. As was the case for the ventral subiculum, glucocorticoid responses to restraint are exacerbated by prefrontal cortex lesions, but ether-induced secretion is unaffected (Diorio et al., 1993). Implants of glucocorticoids in this region inhibit stress-induced HPA activation, suggesting a role for this region in some aspects of glucocorticoid negative feedback (Diorio et al., 1993). However, the overall role of the prefrontal cortex in HPA regulation is not completely clear, as a recent study suggests that medial prefrontal cortex damage reduces stress-induced corticosterone secretion and autonomic activation (Sullivan and Gratton, 1999). Furthermore, frontal cortex stimulation increases corticosterone secretion in anesthetized preparations (Feldman and Conforti, 1985).

2.3. Medial amygdala

Recent anatomical data indicate that the PVN receives little direct input from the amygdala. Among amygdalar subnuclei, the medial amygdala is in the best position to directly interact with the peri-PVN region. The medial nucleus heavily innervates the anterior hypothalamus, largely avoiding the PVN proper, and a portion of this innervation targets the peri-PVN region (see Canteras et al., 1995). Unlike ventral subiculum afferents, medial amygdala terminals are not particularly enriched in the peri-PVN zone relative to innervation of other hypothalamic regions. Indeed, the medial amygdala has heavy projections to the anteroventral periventicular, medial preoptic and anterior hypothalamic nuclei, as well as all known PVN-projecting subnuclei of the bed nucleus of the stria terminalis (posterior intermediate, anterior medial, ventral medial and ventral lateral regions) (Canteras et al., 1995). Indeed, appositions between terminals of medial amygdalar neurons and somata and dendrites of PVN-projecting neurons resident in the bed nucleus of the stria terminalis and medial preoptic area have been observed (Prewitt and Herman, 1998), supporting the existence of disynaptic connections between the two structures.

In general, the amygdala appears to have an excitatory influence on the HPA axis (Herman and Cullinan, 1997). Large lesions of the amygdala block HPA activation following some, but not all, stressors (Allen and Allen, 1974; Feldman and Conforti, 1981; Feldman et al., 1994), and stimulation of amygdalar subnuclei, including the medial nucleus, can elicit corticosterone secretion in anesthetized

preparations (Dunn and Whitener, 1986). Lesions of the medial (but not central) amygdala attenuate PVN c-Fos activation following restraint (Dayas et al., 1999), supporting an excitatory role for this region in responses to emotional stressors. The vast majority of medial amygdalar neurons are GAD-positive (Swanson and Petrovich, 1998); as such, these neurons may excite the PVN through a GABA–GABA disinhibitory process.

Note that other subnuclei, such as the medial component of the central nucleus and the basomedial nucleus, have the potential to interact with the PVN in a di- or polysynaptic manner (e.g., via the bed nucleus of the stria terminalis or the nucleus of the solitary tract; Prewitt and Herman, 1998; Schwaber et al., 1982). However, the central and basomedial nuclei do not project to the peri-PVN region and have minimal direct interaction with parvocellular PVN neurons. The role of the central nucleus in HPA secretion varies substantially across studies (Beaulieu et al., 1986; Dayas et al., 1999; Feldman et al., 1994; Prewitt and Herman, 1997; Van de Kar et al., 1991).

2.4. Lateral septum

The lateral septum sends substantial projections to the region of the PVN, including the adjacent perifornical region and the subparaventricular zone, as well as to the entire anterior hypothalamus (Risold and Swanson, 1997) (Fig. 1). This input is predominantly from ventral subregions of the lateral septum (Risold and Swanson, 1997). In addition to the peri-PVN zone, the ventrolateral septum also projects to PVN-projecting regions of the dorsomedial hypothalamus and medial preoptic area (Risold and Swanson, 1997). Like the medial amygdala, the lateral septum has a predominantly GABAergic phenotype (Risold and Swanson, 1996).

The functional role of the septum in HPA integration is presently obscure. Previous reports indicated that septal damage increases HPA stress responsivity (Dobrakovova et al., 1982; Seggie, 1987; Usher et al., 1974). However, these studies used electrolytic lesion techniques that may compromise hippcocampal or subicular fibers descending through the region, and thus confound interpretation viz. the actual role of the septal nucleus. In addition, most lesions compromise large portions of the septal nucleus, including parts that may not be related to HPA integration. Given our present knowledge of the anatomy of septal–PVN connections, the contribution of the septum to HPA integration is in need of reevaluation.

2.5. Paraventricular thalamus

The paraventricular thalamus sends heavy projections to the subparaventricular zone (Moga et al., 1995). This nucleus also has intense interaction with the SCN and has been therefore implicated in regulation of biological rhythms.

Recent data suggest that the paraventricular thalamus is intimately involved in stress sensitization, a process whereby chronic exposure to a single stressor potentiates HPA activation in response to novel stimuli. The paraventricular thalamus is differentially responsive to novel stressors in the sensitization paradigm, and lesion of the paraventricular thalamus can block the sensitization process (Bhatnagar and Dallman, 1998). Transmission of sensitization may involve cholecystokinin terminals in the paraventricular thalamus (Bhatnagar et al., 2000).

3. Differential innervation of the PVN and its surround by other brain regions regulating HPA activity

There are additional brain regions implicated in HPA function that project differentially to the peri-PVN region, but are not by definition ‘limbic.’ These include the following.

3.1. Suprachiasmatic nucleus

The SCN intensely innervates the subparaventricular zone (Watts et al., 1987). The SCN projection prominently involves vasopressin neurons (Buijs et al., 1998). The SCN is also rich in GABAergic neurons, and these populations are regulated by chronic stress (Bowers et al., 1998), suggesting the possibility of direct or indirect interaction of GABA neurons with the subparaventricular zone. Unlike other areas under discussion, the SCN has direct projections to the PVN; however, terminals are largely relegated to the dorsal and lateral parvocellular regions (Vrang et al., 1995; Watts et al., 1987), which contain primarily neurons that regulate autonomic functions. Despite limited SCN innervation of medial parvocellular neurons, electrical and chemical stimulation of the SCN results in GABAergic and glutamatergic responses in PVN neurons (Hermes et al., 1996; Buijs et al., 1998), including in neurons that express electrical properties characteristic of the parvocellular neurosecretory neurons (Luther et al., 1999).

The SCN is the primary integrator of circadian rhythms; in keeping with this notion, the SCN controls diurnal corticosterone secretion (Cascio et al., 1987). However, recent studies indicate an important role of the SCN in stress integration as well. Lesions of the SCN increase corticosterone secretion in response to stress, supporting an inhibitory role for the SCN in this process; evidence suggests that this activity may be mediated by vasopressinergic interactions with PVN-projecting GABAergic neurons (Buijs et al., 1993; Hermes et al., 2000; Kalsbeck et al., 1992).

3.2. Ascending brainstem pathways

While distinct from limbic PVN projections, ascending afferents from the brainstem appear to also project to the peri-PVN zone. Notably, pontine cholinergic and serotonergic afferents preferentially innervate the peri-PVN region relative to the medial parvocellular PVN, with a particularly prominent input to the subparaventricular zone (Sawchenko

et al., 1983). These classical neurotransmitter pathways are intimately involved in awareness and arousal, and both of these systems have excitatory effects on HPA activity (see (Feldman et al., 1995; Grossman et al., 1993; Herman et al., 1996; Jones and Gillham, 1988)). Thus, the possibility exists that classical neurotransmitter effects on the HPA are mediated (or mitigated) by intervening local circuit neurons. Excitatory effects may thus be relayed by local glutamate neurons or perhaps, in the case of serotonin, through inhibition of GABAergic afferents.

Note that the cholinergic and serotonergic innervation of the PVN region contrasts markedly with that of the noradrenergic and adrenergic systems. Noradrenergic and adrenergic fibers from the A1/C1 and A2/C2 cell groups preferentially innervate the PVN itself, with very sparse innervation of the surrounding region (Cunningham and Sawchenko, 1988; Cunningham et al., 1990). It is thought that these afferents transmit a direct activational stimulus to hypophysiotropic CRH neurons (Plotsky et al., 1989), although recent in vitro electrophysiological evidence suggests that noradrenergic activation of PVN parvocellular neuroendocrine cells may be relayed via intranuclear glutamate circuits (see below; Daftary et al., 2000).

3.3. Other hypothalamic nuclei

There are other regions that project exclusively or differentially to the peri-PVN region. These include the ventromedial nucleus and components of the anterior hypothalamic nucleus, both of which are predominantly GABAergic in phenotype (Canteras et al., 1995; Risold et al., 1994). It would be predicted that these regions function to disinhibit CRH neuronal activity.

3.4. The PVN microenvironment: GABA and glutamate receptors

The possible role of local circuit neurons in parvocellular PVN regulation is supported by data on glutamate and GABA receptor localization. A complex glutamate receptor configuration has recently been demonstrated in the PVN region (Herman et al., 2000). Both the parvocellular PVN and surround contain NMDA1, 2A and 2B receptor mRNA and protein, suggesting that glutamate input can be registered by NMDA receptors in these regions. Interestingly, the configuration of AMPA and kainate receptor subunits is significantly more specialized. In general, the medial parvocellular PVN expresses low levels of AMPA receptor subunits. AMPA receptor subunit expression (particularly GluR2 and GluR4) in the peri-PVN region greatly exceeds that seen in the PVN itself, suggesting that these receptors may be more important in integration of limbic inputs than in direct modulation of PVN activity. The kainate receptors are even more heterogeneous; KA2 mRNA and protein is dispersed throughout the PVN, GluR5 mRNA appears to be preferentially localized to the CRH-containing region of the medial parvocellular zone, and GluR6 and GluR7 are differentially expressed in peri-PVN neurons (Herman et al., 2000). These data indicate that kainate receptors may play a role in selectively regulating parvocellular PVN activation through GluR5. Kainate receptors are well positioned to integrate descending limbic glutamate input in the peri-PVN region as well.

Importantly, PVN-projecting cell populations coexpress glutamate receptors and GAD mRNA, supporting the hypothesis that peri-PVN GABAergic neurons can integrate glutamatergic neurotransmission (Fig. 2). As such, these neurons are in prime position to translate glutamatergic inputs from regions such as the ventral subiculum into inhibition of the PVN.

Similar to glutamate receptors, recent data have revealed that GABA receptors are expressed in the parvocellular

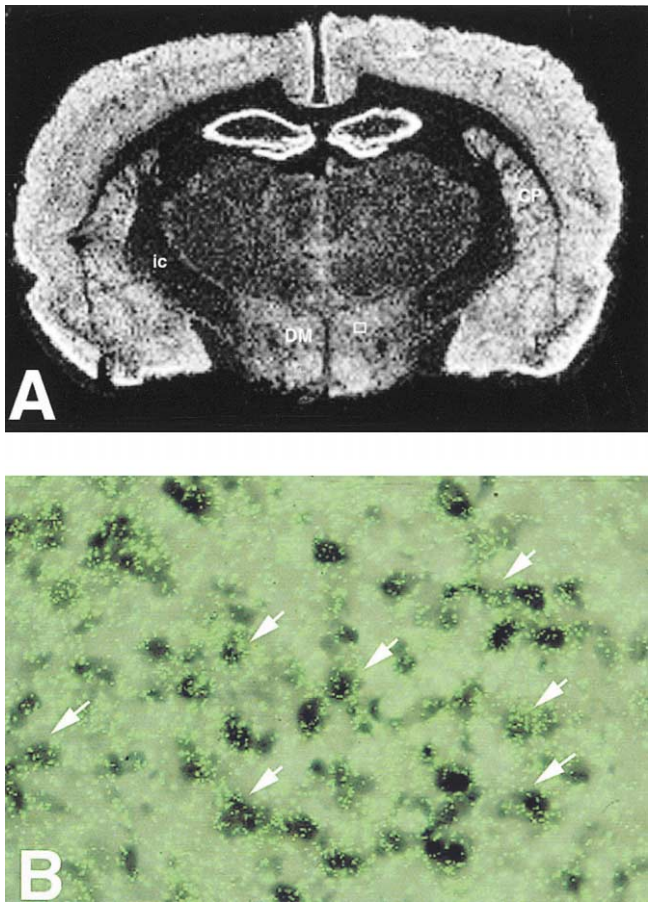


Fig. 2. (A) Darkfield micrograph illustrates the labeling pattern for mRNA transcripts encoding the GluR2 receptor from a coronal section of rat brain at the level of the dorsomedial hypothalamic nucleus. (B) Dual hybridization histochemical demonstration of double-labeled neurons (arrows) with a nonisotopic probe for GAD mRNA (dark cells) and an isotopic probe for GluR2 mRNA (white grains). Photomicrograph was taken from the region indicated by the white box in (A). The data indicate that glutamate-responsive dorsomedial hypothalamic nucleus neurons synthesize GABA, and thus provide evidence for translation of excitatory input into inhibitory output in this region. Abbreviations: CP, caudate putamen; DM, dorsomedial hypothalamic nucleus; ic, internal capsule.

PVN and adjacent territories (Cullinan, 2000; Cullinan and Kelley, 2000). $GABA_A$ receptor mRNAs specific for the $\alpha 1-2$, $\beta 1-3$ and $\gamma 1-2$ subunits have been found to be expressed within hypophysiotropic CRH neurons in the PVN, as well as within the peri-PVN zone. These receptors are also variably expressed within additional intrahypothalamic regions that send local projections to the parvocellular PVN (medial preoptic area, dorsomedial hypothalamic nucleus), supporting the view that these areas serve as GABA-to-GABA relays capable of disinhibiting CRH cells. Interestingly, exposure to chronic (nonhabituating) stress resulted in a down-regulation of transcripts encoding the $\beta 1$ and $\beta 2$ subunits of the receptor in the parvocellular PVN without compensatory changes noted in the $\beta 3$ subunit, suggesting the possibility that $GABA_A$ receptors might be decreased in CRH-containing cells, or alternatively, that changes in subunit composition may occur that alter $GABA_A$ receptor efficacy at this key regulatory site (Cullinan and Wolfe, 2000).

In addition to $GABA_A$ receptors, recent studies have also revealed that $GABA_B$ receptors are expressed in the parvocellular PVN (Margeta-Mitrovic et al., 1999). The role of these receptors in stress regulation has yet to be extensively investigated.

4. Regulation of neuroendocrine PVN neurons by local synaptic circuits

The importance of local GABA and glutamate synaptic circuits in the regulation of the HPA axis is highlighted by physiological analysis of synaptic transmission in the PVN. Consistent with electron microscopic studies showing that the preponderance of synapses in the medial parvocellular PVN, as in the PVN as a whole, are GABAergic and glutamatergic (Decavel and Van Den Pol, 1992), in vitro intracellular recordings demonstrate that most, if not all, of the fast inhibitory and excitatory synaptic inputs to PVN neurons are mediated by GABA and glutamate release, respectively (Wuarin and Dudek, 1991; Boudaba et al., 1996). Studies employing focal stimulation of areas around the PVN have shown that a significant proportion of the GABAergic and, to a lesser degree, the glutamatergic synaptic inputs to PVN neurons derive from local circuit neurons located in perinuclear areas (Tasker and Dudek, 1993; Boudaba et al., 1996, 1997). Thus, chemical microstimulation localized respectively to the perifornical region of the hypothalamus, the anterior bed nucleus of the stria terminalis, the dorsomedial hypothalamus, the anterior hypothalamus and the SCN activated inhibitory synaptic inputs to PVN neurons (Fig. 3). The inhibitory inputs were blocked with a $GABA_A$ receptor antagonist, indicating that they were generated by the activation of presynaptic GABA neurons in these areas (Boudaba et al., 1996). The perinuclear areas in which the presynaptic GABA neurons are localized form a ring around the PVN (Fig. 3), which, as described above, corresponds to the anatomical localization of limbic afferent terminal fields.

Importantly, microstimulation experiments have shown that several of these same perinuclear areas also provide excitatory synaptic inputs to PVN neurons, although the local excitatory circuits are less dense than the inhibitory circuits (Boudaba et al., 1997). Thus, focal stimulation of the dorsomedial hypothalamus, the anterior hypothalamus and the perifornical area elicited excitatory postsynaptic potentials (EPSPs) in PVN neurons (Fig. 3). The EPSPs were blocked by ionotropic glutamate receptor antagonists indicating that they were mediated by glutamate release from glutamatergic neurons located in these perinuclear areas (Boudaba et al., 1997). These data suggest that local circuit neurons may also participate in transneuronal excitation of the PVN. Thus, the immediate surround of the PVN appears to consist of intermixed populations of glutamate and GABA neurons that send local excitatory and inhibitory projections to PVN neurons, including PVN parvocellular neurosecretory neurons (Luther et al., 1999).

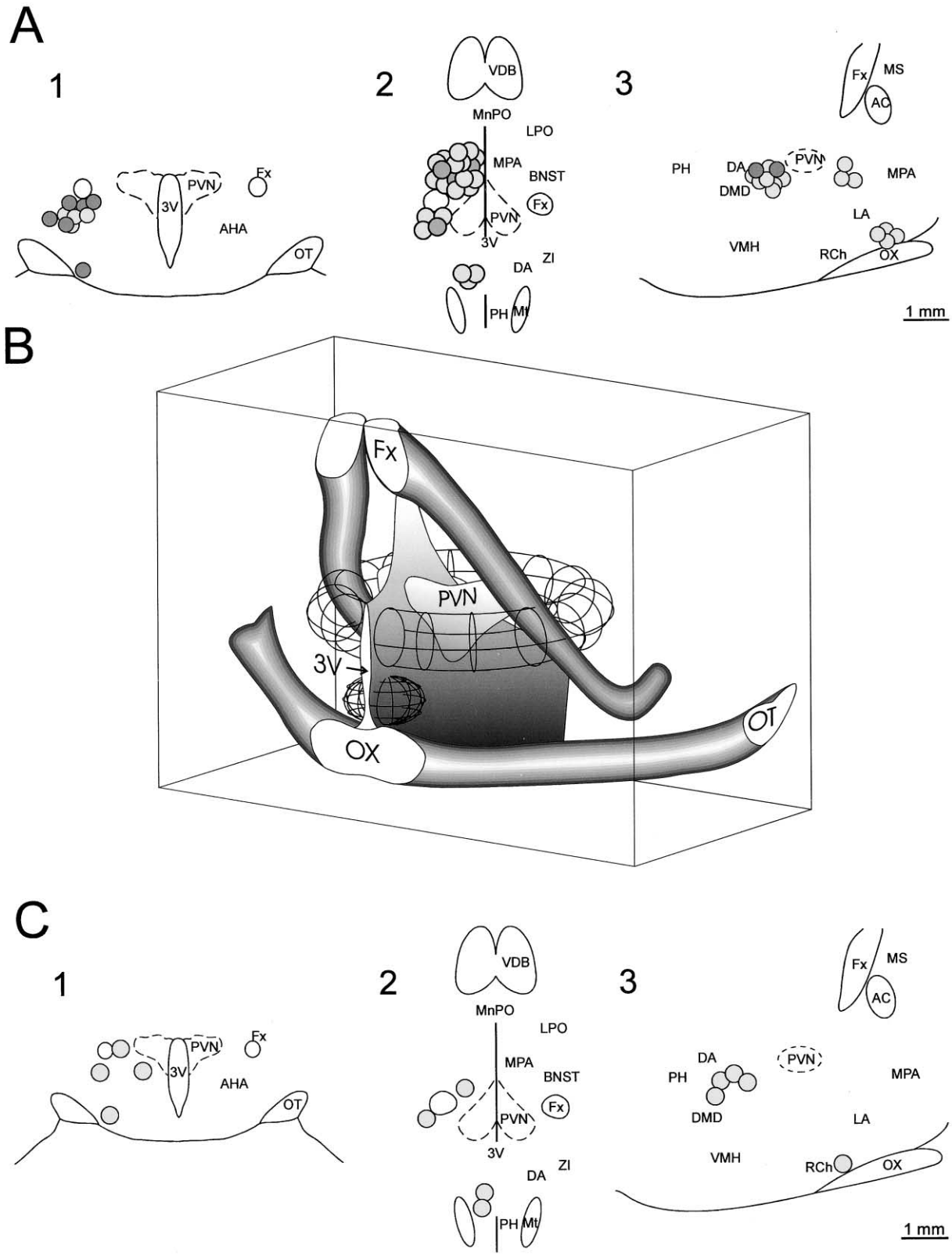
A recent in vitro electrophysiological study suggests that the well-known excitation of PVN neurons by ascending noradrenergic afferents involves a local glutamatergic relay in the hypothalamus (Daftary et al., 2000). Whereas direct noradrenergic excitation of PVN parvocellular neurons was rarely seen in this study (i.e., in $<2\%$ of recorded cells), a substantial percentage of the parvocellular neurons recorded responded to norepinephrine with an increase in spike-dependent, glutamatergic EPSPs. It is not yet clear whether these glutamate neurons are included among the perinuclear local circuit neurons described above, or are located inside the PVN, as has been shown for glutamate neurons projecting to PVN magnocellular neurons (Daftary et al., 1998).

5. Regulation of the stress response by peri-PVN neurons

Peri-PVN GABA and glutamate circuits are clearly capable of modulating HPA activity. Previous studies indicate that local administration of glutamate into the PVN promotes

Fig. 3. Topographic distribution of peri-PVN sites in which focal stimulation elicited GABAergic and glutamatergic synaptic responses in PVN parvocellular neurons. (A) Each light gray circle represents a site at which glutamate microstimulation elicited an increase in IPSPs in PVN parvocellular neurons in coronal (1), horizontal (2) and parasagittal planes (3). Dark gray circles represent sites at which responses were blocked with the $GABA_A$ receptor antagonist, bicuculline methiodide (30 μM). (B) Three-dimensional rendering of the presynaptic GABAergic sites depicted in the three planes in (A). Presynaptic GABA neurons were located in a ring around the PVN and in the area of the SCN. (C) Each gray circle represents a site at which glutamate microdrop application elicited an increase in EPSPs in PVN parvocellular neurons in coronal (1), horizontal (2) and parasagittal planes (3). Figures were adapted from Paxinos and Watson (1986). Figures were reprinted from Boudaba et al., 1996, and Boudaba et al., 1997, with permission.

ACTH release and PVN activation (Darlington et al., 1989; Feldman and Weidenfeld, 1997). Conversely, recent studies indicate that microinfusions of kynurenic acid (a general ionotropic glutamate receptor antagonist) into the PVN inhibit corticosterone responses to restraint stress (Ziegler and Herman, 2000). These data are consistent with direct



glutamatergic excitation of medial parvocellular PVN neurons, and agree with anatomical analyses revealing dense localization of glutamatergic terminals on neurosecretory neurons in the PVN (Decavel and Van Den Pol, 1992).

In contrast, administration of kynurenic acid dorsal to the PVN exacerbates stress-induced corticosterone secretion (Ziegler and Herman, 2000), consistent with removal of glutamate excitation of local inhibitory neurons. These data indicate a glutamate–GABA connection in the region immediately outside the PVN, and are in keeping with the hypothesis that limbic–PVN activation or inhibition is mediated at least in part by local circuit neurons.

Regulation of HPA secretion by GABA has been verified by studies showing that local application of the $GABA_A$ receptor agonist muscimol (900 pmol) reduces corticosterone secretion following restraint stress. These data indicate that, as suggested by the electrophysiological data, GABA-mediated inhibition of the PVN is transduced by $GABA_A$ receptors. Interestingly, application of a fivefold higher dose of muscimol reverses inhibition of the stress response. This effect may be due to spread of the injection to neighboring regions, where $GABA_A$ receptor activation may inhibit local GABA neurons projecting into the PVN (Cullinan, 1998).

6. Excitatory and inhibitory integrations in local hypothalamic–basal forebrain stress relays

Local intrahypothalamic and basal forebrain connections have regulatory effects on the HPA axis via interaction with the PVN. For example, neurons in the PVN-projecting ventrolateral region of the dorsomedial nucleus coexpress GAD and *c-Fos* mRNAs following stress exposure (Cullinan et al., 1996). The vast majority of PVN-projecting neurons in this region are GAD-positive, suggesting inhibitory actions on ACTH release (Cullinan et al., 1996). As noted above, glutamate microstimulation of the dorsomedial nucleus (particularly its rostral aspect) activates predominantly an inhibitory input to PVN neurons, consistent with this role (Boudaba et al., 1996, 1997). However, other studies indicate that injection of muscimol into the dorsomedial nucleus attenuates ACTH release following stress, suggesting removal of an excitatory projection to the PVN (Stotz-Potter et al., 1996). The coexistence of an excitatory pathway alongside the inhibitory pathway from the dorsomedial nucleus to the PVN is further supported by evidence that local microstimulation of the dorsomedial nucleus with glutamate receptor agonists sometimes elicits EPSPs in vitro (Boudaba et al., 1997) and enhances ACTH release in vivo (Bailey and Dimicco, 2001). Thus, the dorsomedial nucleus may play a role in both excitation and inhibition of the HPA axis, either via excitatory input to the PVN or disynaptic interneuronal disinhibition. The latter is an intriguing possibility, particularly given that regions targeted for microinfusions were considerably medial to the bulk of direct PVN-projecting, stress-activated dorsomedial nucleus neurons (Cullinan et al., 1996; Stotz-Potter et al., 1996). The net effect of dorsomedial nucleus activation on the PVN may thus involve selective activation of excitatory vs. inhibitory PVN afferents by limbic forebrain and perhaps brainstem pathways.

Notably, the dorsomedial nucleus shows stimulus-specific patterns of c-*fos* induction following stress. Air-puff startle or open-field exposure induces substantially more extensive c-*fos* mRNA labeling in the dorsomedial nucleus than either hemorrhage or ether (Emmert and Herman, 1999; Thrivikraman et al., 2000). Differential c-*fos* induction occurs despite comparable or greater levels of ACTH and corticosterone secretion by systemic stressors (Emmert and Herman, 1999; Thrivikraman et al., 2000), indicating that the effects on c-*fos* expression are not due to greater PVN activation by startle or novelty. Interestingly, injection of muscimol into the dorsomedial nucleus blocked PVN c-*fos* induction to air-puff startle but not hemorrhage (Morin et al., 2001), further consistent with selective involvement in processing of psychogenic stressors. These data parallel studies showing stress-specific inhibition of the HPA by the hippocampus, and suggest that local circuit neurons in the dorsomedial nucleus may be a component of the multisynaptic ventral subiculum–PVN circuit.

Parvocellular PVN outflow also appears to be regulated by neurons in the medial preoptic area. Lesions of the medial preoptic area increase HPA stress responsiveness, consistent with inhibitory input to the PVN (Viau and Meaney, 1996). Stress-activated neurons in the preoptic area have direct projections to the PVN; these neurons coexpress GAD, consistent with direct GABAergic innervation of PVN neurons (Cullinan et al., 1996). Neurogenic stimuli increase firing rate of preoptic nucleus neurons (Saphier et al., 1988), consistent with stress activation. However, high-frequency stimulation of this region can enhance or inhibit PVN electrical activity and increase corticosterone release (Saphier and Feldman, 1986), suggesting that like the dorsomedial nucleus, the preoptic area may be involved in both excitatory and inhibitory aspects of PVN regulation. Notably, the sites of electrical stimulation are generally more medial than stress-activated PVN-projecting preoptic neurons (Cullinan et al., 1996; Saphier and Feldman, 1986), indicating that more work is needed to determine whether excitatory and inhibitory inputs arise from anatomically distinct subregions of the nucleus.

The bed nucleus of the stria terminalis also shows a functional heterogeneity of interaction with the HPA axis. However, in this case, differential effects are segregated in accordance with projection pathways of neuronal subpopulations. For example, lesions of the posterior intermediate subnucleus, which is rich in PVN-projecting neurons (Fig. 1), increase CRH mRNA expression (Herman et al., 1994), whereas stimulation of this region decreases corticosterone secretion (Dunn, 1987). Conversely, stimulation of lateral regions, which have substantially less direct input to the PVN, increases corticosterone levels (Dunn, 1987), whereas lesions inhibit CRH mRNA expression (Herman

et al., 1994), block corticosterone responses to olfactory stimuli (Mor et al., 1987) or amygdala stimulation (Feldman et al., 1990) and attenuate corticosterone and ACTH responses following fear conditioning (Gray et al., 1993). As was the case with the dorsomedial and preoptic nuclei, regulation of the PVN by the bed nucleus of the stria terminalis appears compartmentalized, with inhibitory and excitatory projections in close anatomical proximity.

Thus, it is evident that local intrahypothalamic and basal forebrain neurons play a major role in integration of PVN stress responses. These neurons likely translate descending information from limbic structures into net excitation or inhibition of the PVN in an anatomically precise manner.

7. Limbic gating at the PVN: weighing the limbic system in the balance?

Anatomical and physiological data indicate that descending limbic system neurons are in position to modulate the activity of hypophysiotrophic PVN neurons at the local level. In many cases, the peri-PVN projection relays are likely to reverse the valence of descending signaling systems (Fig. 4). For example, in terms of the ventral subiculum, this anatomical arrangement allows a presumptive excitatory glutamate projection to initiate inhibition via GABAergic interneurons. In the case of the medial amygdala, a GABAergic projection to local GABAergic projections may disinhibit the PVN. The proximity of these cell populations to the PVN suggests that they function as a gateway for descending limbic system modulation of adrenocortical secretion.

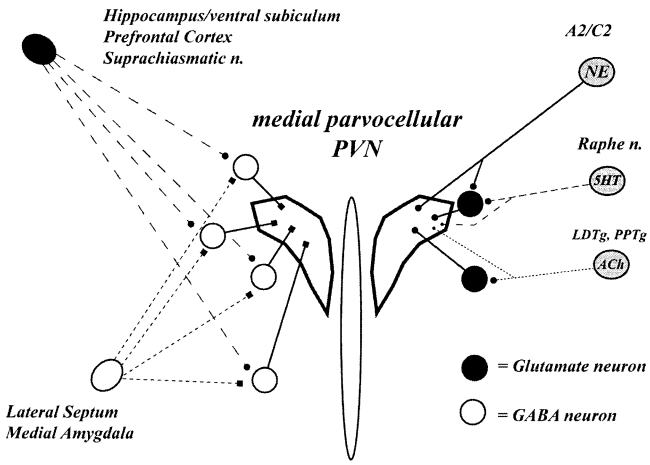


Fig. 4. Schematic representation of possible limbic system interactions with hypophysiotrophic medial parvocellular PVN neurons. Glutamate outflow from regions such as the ventral subiculum and perhaps prefrontal cortex synapse on local GABAergic neurons (Cullinan et al., 1993), producing a net inhibition of PVN neurons. In contrast, GABAergic afferents from regions such as the medial amygdala and perhaps lateral septum may inhibit PVN projecting GABA cells, resulting in disinhibition. In contrast to limbic projections, ascending brainsteam cell groups have direct projections into the medial parvocellular PVN (Swanson and Sawchenko, 1983); in the case of acetylcholine and serotonin, these inputs are weak in comparison to innervation of the PVN surround (Sawchenko et al., 1983), raising the possibility that these projection systems work through intermediary glutamate neurons know to be present in these areas (Tasker et al., 1998). Note that limbic afferents may also have opportunity to interact with these glutamate cells (not shown).

Local circuit neurons likely represent one aspect of limbic–PVN interaction. All the limbic regions noted have extensive projections to other PVN-projecting hypothalamic nuclei, including the dorsomedial nucleus, medial preoptic area, lateral hypothalamic area, arcuate nucleus and mammillary nuclei. These regions are integral to homeostasis, controlling cardiovascular tone, energy balance and cytokine signaling. As such, limbic projections are positioned to integrate cognitive signals with hypothalamic function, providing an opportunity for the PVN effector system to generate an HPA output appropriate to both cognitive and physiological status.

It is important to note that local circuit integration of PVN limbic input may also have an impact on other cell populations in the PVN, most notably magnocellular vasopressin and oxytocin neurons and preautonomic neurons regulating cardiovascular tone (Swanson and Sawchenko, 1983). In many cases, input to these cell groups parallels innervation of the medial parvocellular PVN (Swanson and Sawchenko, 1983); in particular, it is likely that neurons in the peri-PVN region also innervate magnocellular populations (Roland and Sawchenko, 1993). While the role of magnocellular and preautonomic neurons in HPA stress responses is ill-defined, it is clear that vasopressin and oxytocin secretion is elicited in a stressor-specific manner (Gibbs, 1984; Plotsky et al., 1985; Romero et al., 1993) and may comprise a component of the integrated neuroendocrine response to stressful stimuli. This component also stands to be regulated by local circuit integrative mechanisms.

The presence of the intervening synapse between limbic projections and parvocellular (or magnocellular) PVN effector neurons has several functional implications. Integration proximal to the level of the PVN may be an important regulatory checkpoint in HPA integration, as it represents an opportunity for hierarchical organization of synaptic input in order of importance. For example, information on cardiovascular status is of high survival relevance, in that prompt activation of the HPA axis is an important component of physiological reactions to cardiovascular collapse. Accordingly, regions regulating cardiovascular tone (e.g., nucleus of the solitary tract) project directly into the medial parvocellular PVN. Similarly, input from hypothalamic homeostats is also high priority, as the HPA system contributes to redistribution of bodily resources in times of physiologic need. Limbic information is a level removed from emergency; in fact, it provides an anticipation or expectation of an outcome (e.g., novel environment) that may or may not require glucocorticoid mediation, rather than one that clearly requires an immediate HPA response (e.g., hypoglycemia). In addition, limbic input may have contradictory or conflicting implications for the organism, e.g., the hippocampal

(inhibitory) and amygdalar (excitatory) systems appear to relay different types of information to the PVN region. The intervening relay neuron is therefore in good position to summate concurrent activation of these systems into an appropriate net signal to the PVN neuron. Thus, the local synapse provides a gateway for descending afferent systems, allowing it to be evaluated with respect to physiological status as well as activity in other PVN regulatory pathways.

Functional gating of limbic information by local PVN-projecting neurons may play a role in the genesis of HPA dysfunction seen in aging and affective disease. For example, both situations are marked by hippocampal dysfunction and HPA disinhibition (Bremner et al., 2000; Dodt et al., 1991; Kathol et al., 1989; Lupien et al., 1999; Sapolsky, 2000; Sapolsky et al., 1986). Hippocampal impairment stands to generate a functional loss of subicular inhibition of PVN neurons. The loss of glutamate input to peri-PVN GABAergic connections may effectively remove a brake on the HPA axis, resulting in a net overactivation of the HPA system. Alternatively, overdrive of HPA excitatory circuitry, such as that emanating from the amygdala, may overcome inhibitory input to the PVN and permit an increase in the net excitability of the system. The view outlined here suggests that local circuit neurons integrating limbic convergence will serve as fruitful targets for investigation of neuroendocrine deficits associated with human aging and affective disease.

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