Ascending mechanisms of stress integration: implications for brainstem regulation of neuroendocrine and behavioral stress responses

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Abstract

In response to stress, defined as a real or perceived threat to homeostasis or well-being, brain systems initiate divergent physiological and behavioral processes that mobilize energy and promote adaptation. The brainstem contains multiple nuclei that engage in autonomic control and reflexive responses to systemic stressors. However, brainstem nuclei also play an important role in neuroendocrine responses to psychogenic stressors mediated by the hypothalamic-pituitary-adrenocortical axis. Further, these nuclei integrate neuroendocrine responses with stress-related behaviors, significantly impacting mood and anxiety. The current review focuses on the prominent brainstem monosynaptic inputs to the endocrine paraventricular hypothalamic nucleus (PVN), including the periaqueductal gray, raphe nuclei, parabrachial nuclei, locus coeruleus, and nucleus of the solitary tract (NTS). The NTS is a particularly intriguing area, as the region contains multiple cell groups that provide neurochemically-distinct inputs to the PVN. Furthermore, the NTS, under regulatory control by glucocorticoid-mediated feedback, integrates affective processes with physiological status to regulate stress responding. Collectively, these brainstem circuits represent an important avenue for delineating interactions between stress and health.

1. Introduction

In response to real or perceived threats to homeostasis or well-being, an organism generates multiple integrated stress responses that provide resources for physiological and behavioral adaptation (de Kloet et al., 2005; Myers et al., 2014b). Physiological stress responses promote energy mobilization and redistribution through two primary systems, the autonomic

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nervous system and the hypothalamic-pituitary-adrenocortical (HPA) axis (for review see Ulrich-Lai and Herman, 2009). Autonomic responses to stress increase heart rate, blood pressure, and glucose availability, providing energetic substrates for stress adaptation (Ulrich-Lai and Herman, 2009). The neuroendocrine HPA axis causes the secretion of glucocorticoids (principally corticosterone in rodents and cortisol in humans) from the adrenal cortex (Keller-Wood and Dallman, 1984). Activity of the HPA axis is initiated by parvocellular corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus of the hypothalamus (PVN). At the level of the anterior pituitary, CRH leads to the systemic release of adrenocorticotropic hormone (ACTH), the primary stimulus for glucocorticoid secretion (Ulrich-Lai and Herman, 2009). Glucocorticoids then signal throughout the body to regulate many systemic and neural functions, including hepatic glycogenolysis and neuronal metabolism (Herman, 2013; Herman et al., 2003). Behavioral stress responses depend on a large network of brain systems involved in appraisal, emotion, and memory (Joëls and Baram, 2009; Sousa and Almeida, 2012). Behavioral-regulatory circuits include forebrain sites such as the amygdala, prefrontal cortex, and hippocampus; importantly, these networks also interact with the hypothalamus and brainstem to integrate behavioral responses with HPA axis and autonomic activity (Joëls and Baram, 2009; McKlveen et al., 2015; Ulrich-Lai and Herman, 2009).

Aberrant activation of the HPA axis plays a role in many psychiatric illnesses including depression, anxiety, and post-traumatic stress disorder (for review see Jacobson, 2014; Ströhle and Holsboer, 2003). Glucocorticoids also exert profound effects on somatic health, particularly cardio-metabolic processes (Vogelzangs et al., 2010). In fact, cardiomyocyte glucocorticoid signaling is essential for maintaining cardiac function and survival (Oakley et al., 2013). Glucocorticoids also act in the hindbrain to increase arterial pressure and modulate baroreflex control (Bechtold and Scheuer, 2006; Scheuer et al., 2007). Therefore, the ascending brainstem circuits that regulate HPA axis activity and stress-related behaviors represent an important avenue for delineating the complex relationships between stress, behavior, and health.

The current review considers the key brainstem nuclei providing direct input to the PVN, circuits that have predominantly been mapped in rodents. We discuss their connectivity, chemistry, and role in HPA axis and behavioral stress responses, principally anxiety-, fear-, and depression-related behaviors. Although the autonomic nervous system mediates many important aspects of stress responsiveness, the role of the brainstem in autonomic regulation has been described elsewhere (see Ally, 1998; Bandler et al., 2000; Scislo and O'Leary, 2005 for review). Therefore, the current review will focus on the ascending pathways to the endocrine hypothalamus and their effects on behavior. The brainstem also gives rise to neuromodulatory projections that collateralize throughout the brain. These primarily aminergic systems regulate neural and behavioral function throughout the limbic system and cortex. Cataloging the diversity of these functions is beyond the scope of a single review; thus, we will provide references, where applicable, to reviews that focus on specific messenger systems and their effects on behavior mediated through interactions with the forebrain. Brainstem projections to the forebrain also have indirect effects on HPA axis stress responses via neuromodulation within limbic structures (Radley et al., 2008). These multisynaptic network-level mechanisms have yet to be unraveled; consequently, we will

focus on the monosynaptic brainstem inputs to the PVN. Our review outlines brainstem nuclei that directly regulate the HPA axis, from rostral to caudal these are the periaqueductal gray (PAG), raphe nuclei, parabrachial nuclei (PBN), and locus coeruleus (LC). Additionally, we will explore the nucleus of the solitary tract (NTS) in detail and its rich collection of messengers that interact with glucocorticoids, including norepinephrine, glucagon-like peptide-1 (GLP-1), and glutamate. Finally, we will discuss how recent advances have expanded our understanding of the role of ascending brainstem projections, suggesting that these circuits form a critical hub for integrating interoceptive input with descending limbic information to coordinate endocrine and behavioral stress responses.

2. Brainstem inputs to the paraventricular hypothalamus (PVN)

The PVN is composed of a relatively small number of neuropeptide-containing cells that regulate many aspects of endocrine and homeostatic function (for reviews see Herman et al., 2002a; Herman et al., 2002b). Although some PVN neurons project within the central nervous system and act in a pre-autonomic fashion, other PVN cell groups are neuroendocrine (Biag et al., 2012; Hallbeck and Blomqvist, 1999). Thus, multiple subregions of the PVN are defined based on neurochemistry and connectivity (for reviews see Herman et al., 2008; Swanson and Sawchenko, 1983). Generally, the PVN is divided into magnocellular and parvocellular regions. For instance, the anterior, medial, and posterior magnocellular portions of the PVN contain neurons that synthesize oxytocin and vasopressin for release from neurosecretory terminals in the posterior pituitary (van Leeuwen et al., 1979). Within parvocellular regions, the lateral, dorsal, and ventromedial areas give rise to central projections that target the basal forebrain, brainstem, and spinal cord (Swanson and Sawchenko, 1983). These central circuits regulate numerous autonomic and behavioral processes through the release of oxytocin, vasopressin, and other transmitters (Knobloch and Grinevich, 2014). Importantly, the dorsomedial and anterior divisions of parvocellular neurosecretory cells project to the median eminence where they secrete CRH, the primary ACTH secretagogue, as well as vasopressin, which acts synergistically with CRH to promote HPA axis activity (Sawchenko et al., 1984; Vale et al., 1981). Thus, the HPA axis response to stress originates in a circumscribed collection of neurosecretory cells in the PVN (Ulrich-Lai and Herman, 2009). These cells express a multitude of neurotransmitter and peptide receptors that integrate signals from PVN-projecting circuits. Importantly, the PVN only receives direct synaptic input from a restricted number of brain regions (for review see Herman et al., 2003). These PVN-projecting afferents arise from other hypothalamic nuclei, the bed nucleus of the stria terminalis (BST), and importantly, the brainstem (Fig. 1). The intra-hypothalamic interactions have been described in detail (Cullinan et al., 1996; Myers et al., 2014a; Ulrich-Lai et al., 2011), as have projections from the BST (Choi et al., 2007; Cullinan et al., 1996; Dong and Swanson, 2006; Dong et al., 2001; Radley and Sawchenko, 2011). Briefly, these circuits have been proposed to form a hierarchy for mediating limbic stress integration and regulating HPA axis responses to psychogenic stressors (Herman et al., 2005; Myers et al., 2012). In contrast, brainstem projections have been postulated to mediate reflexive responses to systemic stressors. However, the multiple brainstem nuclei innervating the PVN represent a substantial proportion of stress-regulatory input to CRH neurons (Larsen and Mikkelsen, 1995; Ziegler

et al., 2012). Accordingly, these structures are well-positioned to regulate endocrine responses to both systemic and psychogenic stress, as well as behavior (for review see Morgane and Mokler, 2006; Morgane et al., 2005).

3. Periaqueductal gray (PAG)

The PAG is a midbrain region surrounding the cerebral aqueduct that regulates many vital functions, including analgesia, autonomic responses, and behaviors related to defense, fear, and anxiety (see Bandler and Shipley, 1994; Behbehani, 1995; Carvalho-Netto et al., 2007). The columnar organization within the PAG gives rise to diverse projections with individualized functions (Bandler and Shipley, 1994). Multiple tract-tracing studies have identified specific PAG projections to the PVN arising from the pre-commissural and commissural subdivisions, dorsolateral column, and ventrolateral column (Cameron et al., 1995; Canteras and Goto, 1999; Floyd et al., 1996; Ziegler et al., 2012). Importantly, fibers from the ventrolateral PAG robustly target the medial, dorsal, and anterior parvocellular PVN (Floyd et al., 1996), providing an anatomical substrate for HPA axis regulation. Projections from the lateral PAG to the PVN co-localize with the glutamatergic marker vesicular glutamate transporter 2 (vGluT2) (Ziegler et al., 2012), suggesting the PAG may have an excitatory influence on the HPA axis. Multiple modalities of stress, including predator exposure, loud noise, forced swim, and restraint, increase neuronal activation in the dorsolateral PAG (Campeau and Watson, 1997; Canteras and Goto, 1999; Cullinan et al., 1995). Furthermore, electrical stimulation of the PAG in the open field increases corticosterone, accompanied by flight behaviors (Lim et al., 2011). Interestingly, intra-PAG administration of a cannabinoid receptor agonist also increases plasma corticosterone, yet decreases stress-induced hyper-locomotion and increases freezing (Finn et al., 2004). Thus, there appears to be functional topography of the PAG for regulating fight-or-flight behaviors, with different PAG columns implicated in divergent behavioral strategies. For instance, the ventrolateral PAG may be involved in passive defense strategies (e.g. freezing), while the dorsal PAG may mediate active defense behaviors (e.g. flight) (Monassi et al., 1997).

4. Raphe nuclei

The raphe nuclei are a collection of functionally and anatomically diverse cell groups that span the brainstem and contain the majority of the serotonin-producing neurons in the central nervous system. The organization and physiology of the raphe nuclei are comprehensively reviewed elsewhere (see Jacobs and Azmitia, 1992; Lowry, 2002). In general, the raphe nuclei can be divided into primarily ascending and primarily descending groups. The descending nuclei, including the raphe magnus and raphe pallidus, are spinal-projecting and regulate autonomic functions, among others (Jacobs and Azmitia, 1992). The ascending raphe nuclei, primarily the dorsal raphe (DR) and the median raphe (MnR), send projections throughout the brain and regulate a variety of behaviors related to mood, anxiety, and stress (Jacobs and Azmitia, 1992). The effects of serotonin signaling are dependent not only on the topography of the raphe, but also on a vast array of receptor subtypes. Consequently, serotonin can have divergent and opposing effects on many processes. For detailed reviews of serotonin interactions with the limbic system in behavioral control see Deakin and Graeff, 1991; Hale et al., 2012; Paul et al., Briefly, the MnR is proposed to

inhibit stress responses, while the DR appears to facilitate arousal and anxiety-like behaviors (Lowry, 2002). However, both the MnR and DR send serotonergic fibers to the PVN, although, the majority of serotonergic fibers terminate in the region immediately surrounding the PVN (Sawchenko et al., 1983). Models of altered serotonin signaling, including serotonin transporter knockout mice and serotonin receptor overexpression in the medial hypothalamus, suggest that raphe projections, whether to PVN proper or surrounding areas, likely stimulate stress responses (Hanley and Van de Kar, 2003; Li et al., 2004). Importantly, injections of a serotonin-specific neurotoxin directly into the PVN inhibit stress-induced ACTH release (Jorgensen et al., 1998), indicating that serotonin input to the PVN predominantly excites the HPA axis. Whether PVN-projecting raphe neurons directly regulate behavioral responses to stress is somewhat unclear. The largest numbers of serotonin neurons arise from the DR and ascend through two major fiber tracts, the forebrain bundle tract and the peri-ventricular tract (Hale and Lowry, 2011; Hale et al., 2012). The former targets major limbic nuclei, while the later innervates the hypothalamus; thus, it is possible that behavioral and endocrine regulation may be partially segregated within the raphe nuclei. In support of this, decreased serotonin signaling in the medial hypothalamus attenuates ACTH secretion but not anxiety-like behaviors (Li et al., 2004).

5. Parabrachial nuclei (PBN)

The PBN are located in the reticular formation at the midbrain-pontine junction and are primarily composed of lateral and medial nuclei (Gauriau and Bernard, 2002). The PBN are involved in many homeostatic functions including chemoreception, nociception, and autonomic control (Chamberlin, 2004; Gauriau and Bernard, 2002; Spector, 1995). Although the role of the PBN in stress responding has been understudied, the nuclei project to the hypothalamus and may play a role in neuroendocrine-autonomic integration. Specifically, the lateral nuclei, including central lateral, superior lateral, and the external lateral, densely innervate the PVN (Bester et al., 1997). Neurochemically, the lateral PBN projections to the PVN are glutamatergic and predominantly innervate parvocellular neurons (Krukoff et al., 1992; Ziegler et al., 2012), suggesting a possible excitatory influence on HPA axis reactivity. Neurons of the PBN are activated by both systemic (e.g. visceral illness) and psychogenic (e.g. restraint) stressors, including cells that express peptides such as calcitonin gene-related peptide, neurotensin, and CRH (Kainu et al., 1993). While few rodent studies have examined neuroendocrine regulation by the PBN, CRH injections into the PBN increase circulating ACTH in anesthetized cats (Carlson et al., 1994). Behaviorally, the PBN are largely implicated in aversive behaviors related to interoceptive information, such as conditioned taste aversion (Bester et al., 1997; Reilly and Trifunovic, 2001).

6. Locus coeruleus (LC)

The LC, a discrete pontine nucleus contiguous with the fourth ventricle, houses the majority of the norepinephrine-expressing neurons in the brain and innervates the entire neuraxis (Dahlström and Fuxe, 1964; Schwarz and Luo, 2015; Swanson, 1976). The LC modulates behaviors related to arousal, attention, cognitive flexibility, and stress responses (Cassens et al., 1981; Francis et al., 1999; Valentino and Van Bockstaele, 2008). The LC is also the primary source of norepinephrine for the cortex and hippocampus, representing a major

component of the central arousal network (Valentino and Van Bockstaele, 2008). Thus, LC-corticolimbic interactions are key for behavioral state regulation (see Aston-Jones and Cohen, 2005; Berridge and Waterhouse, 2003 for review). The LC provides sparse direct input to the PVN that is limited to the medial parvocellular division and predominantly, but not exclusively, noradrenergic (Cunningham and Sawchenko, 1988; Sawchenko and Swanson, 1982). The LC is activated by numerous modalities of stress, including psychosocial, physiological, and nociceptive stimuli (Cassens et al., 1981; Francis et al., 1999; McCall et al., 2015; Reyes et al., 2008). Accordingly, the role of the LC in modulating the HPA axis differs depending on the duration and intensity of stressors (Armario et al., 2012). Generally, LC function appears more important for neuroendocrine responses to acute stressors than for repeated challenges following chronic stress. For instance, norepinephrine depletion of the LC by neurotoxic lesions blunts HPA axis responses to acute restraint, but do not prevent the induction of HPA axis hyperactivity following chronic variable stress (Ziegler et al., 1999). Although, repeated or prolonged activation of the LC has been implicated in the development of stress-related behaviors (George et al., 2013; Reyes et al., 2015; Valentino et al., 2012). Specifically, recent studies employing chemogenetic and optogenetic modulation of LC norepinephrine neurons determined the necessity and sufficiency of these cells for stress-induced anxiety-like behavior (McCall et al., 2015). Interestingly, the effects of stress on behavior may be mediated, in part, by CRH signaling in the LC. Although CRH input to the LC has been shown to arise from amygdalar and brainstem afferents (McCall et al., 2015; McFadden et al., 2012), the sensitivity of LC neurons to CRH is not static and is influenced by factors such as prior stress exposure (Curtis et al., 1999, 1995).

7. Nucleus of the solitary tract (NTS)

The NTS is an expansive and neurochemically diverse cell group in the dorsal medulla. The NTS is a critical hub for integrating interoceptive and viscero-sensory input with descending affective and cognitive information from the limbic forebrain (for excellent reviews see (Rinaman, 2011, 2007). The best-described functions of the NTS relate to autonomic control, yet emerging evidence points to NTS involvement in many behavioral and neuroendocrine processes (Rinaman, 2011). This diversity of functions is mediated by an array of neurotransmitters and peptides with partially overlapping expression. For instance, the intermediate NTS contains the A2 group of norepinephrine-producing neurons (Dahlström and Fuxe, 1964). This cell group provides a substantially greater proportion of noradrenergic projections to the PVN than the aforementioned locus coeruleus (i.e. A6), and the overwhelming majority of norepinephrine input to medial parvocellular neurons relative to other noradrenergic populations (e.g. A1) (Sawchenko and Swanson, 1982). In addition to norepinephrine, these neurons also co-release glutamate (Stornetta et al., 2002). Furthermore, NTS noradrenergic neurons contain peptides such as prolactin-releasing peptide and neuropeptide Y, which may act synergistically with norepinephrine on PVN CRH neurons (Chen et al., 1999; Sawchenko et al., 1985; Uchida et al., 2010). A separate cell group located in the caudal NTS that expresses glucagon-like peptide-1 (GLP-1), as well as glutamate (Zheng et al., 2015), also gives rise to PVN projections; importantly, these cells do not produce norepinephrine (Ghosal et al., 2013; Larsen et al., 1997). Neurons

throughout the NTS are activated by a variety of acute and chronic stressors (Flak et al., 2012; Pezzone et al., 1993; Rinaman, 1999). Moreover, a portion of NTS chronic stress-activated neurons are immunoreactive for the norepinephrine-synthesizing enzyme dopamine-β-hydroxylase (DBH) (Flak et al., 2012). Direct stimulation of the NTS, as well as pharmacological activation of NTS-derived norepinephrine and GLP-1 signaling in the PVN, indicate that the nucleus promotes HPA axis activation (Day et al., 1985; Kinzig et al., 2003). In addition to sending projections that target subcortical limbic regions critical for regulating behavioral responses to stress, the NTS also receives direct input from the amygdala, BST, and prefrontal cortex (van der Kooy et al., 1984). Consequently, the NTS has been implicated in behaviors related to anxiety, depression, and fear memory (Ghosal et al., 2014; Miyashita and Williams, 2002).

7.1. Norepinephrine

Housed within the NTS, A2 noradrenergic neurons form a bidirectional interface linking physiological processes with emotional and cognitive events, particularly in response to stressors (Rinaman, 2011; Sawchenko and Swanson, 1982, 1981). These neurons integrate feedback from physiological systems with affective state and relay this information to the PVN, forming a major HPA axis excitatory pathway (Morilak et al., 2005; Plotsky et al., 1989; Rinaman, 2011; Sawchenko and Swanson, 1981; Sawchenko et al., 1996). Specifically, catecholaminergic lesions of NTS neurons attenuate HPA axis responses (Bundzikova-Osacka et al., 2015); further, electrical stimulation of ascending catecholaminergic afferents to the PVN and intracerebroventricular injection of norepinephrine induce CRH secretion in a α1-adrenergic receptor-dependent manner (Plotsky, 1987; Plotsky et al., 1989). Collectively, these data indicate that norepinephrine is both necessary and sufficient for HPA axis activation. Noradrenergic projections to the PVN are particularly important for challenges to physiological homeostasis. In fact, neurochemically specific interventions have illuminated a role for these circuits in facilitating HPA responses to hypotension (Plotsky, 1987), glucoprivation (Ritter et al., 2003), osmotic challenge (Khan et al., 2011), and inflammation (Bienkowski and Rinaman, 2008). However, noradrenergic neurons also activate HPA axis responses to psychogenic stressors, effects that appear to be weighted towards acute reactivity, as opposed to chronic stress (Bundzikova-Osacka et al., 2015). In fact, PVN injections of saporin toxin conjugated to a DBH antibody (a method to remove epinephrine/norepinephrine inputs to PVN) blunt ACTH and corticosterone responses to acute restraint. However, these inputs do not appear to be necessary for glucocorticoid hyper-responsiveness after chronic variable stress (Flak et al., 2014). In contrast, repeated cold stress increases HPA axis responses to a novel stressor through enhanced α1-adrenergic receptor responsiveness in the PVN (Ma and Morilak, 2005), suggesting that the modality of chronic stress (i.e. heterotypic vs. homotypic) may be an important determinant for the involvement of PVN norepinephrine signaling.

7.2. Glucagon-like peptide-1 (GLP-1)

GLP-1 is an incretin hormone most prominently expressed in epithelial L-cells of the intestine (Holst, 2007). The primary systemic role of GLP-1 is post-prandial stimulation of pancreatic insulin production (Holst, 2007). In addition, there are a restricted collection of hindbrain neurons that produce and transmit GLP-1 to homeostatic regulatory areas,

including the PVN. The neuronal form of GLP-1 is derived from differential post-transcriptional processing of preproglucagon (PPG), whereas glucagon is the major product of PPG in the pancreas (Larsen et al., 1997). In the central nervous system, GLP-1 signaling regulates a variety of homeostatic processes, including body weight, blood glucose, heart rate, and blood pressure (reviewed in Ghosal et al., 2013). The vast majority of GLP-1 immunoreactive cells in the hindbrain reside in the visceral NTS, with sparse expression in the ventral and medial reticular nuclei (Han et al., 1986; Jin et al., 1988). This location in the caudal NTS allows GLP-1 neurons to monitor visceral signals and respond to physiological stressors (Larsen et al., 1997; Rinaman, 1999). In addition to activation by systemic/visceral stressors, GLP-1 neurons are also activated by psychogenic stress and have been implicated in anxiety-like behavior (Kinzig et al., 2003). GLP-1 neurons project from the NTS to the hypothalamus where they appose CRH neurons in the medial parvocellular PVN (Sarkar et al., 2003; Tauchi et al., 2008). Furthermore, GLP-1 and vGluT2 co-localize in synaptic terminals targeting the PVN (Zheng et al., 2015). Thus, projections from GLP-1-producing neurons facilitate the HPA axis response to stress. Intracerebroventricular injections of GLP-1 activate the medial parvocellular PVN, an effect prevented by pre-administration of a GLP-1 receptor antagonist (Larsen et al., 1997; Turton et al., 1996). Additionally, GLP-1 injected into the PVN increases plasma ACTH and corticosterone (Kinzig et al., 2003). The HPA axis excitatory effects of GLP-1 in the PVN appear to be divergent from GLP-1 effects on anxiety-like behavior, the later depending on GLP-1 signaling in the amygdala (Kinzig et al., 2003). Interestingly, pharmacological administration of GLP-1 interacts with chronic variable stress to increase sensitization of glucocorticoid responses to a novel stressor (Tauchi et al., 2008), suggesting that GLP-1 may be particularly important for tuning reactivity to chronic stress.

7.3. Glucocorticoid interactions

The glucocorticoid receptor (GR) is expressed in NTS GLP-1 neurons (Härfstrand et al., 1986; Rinaman, 2011) and binds to glucocorticoid response elements upstream of the PPG promoter to regulate PPG transcription (Zhang et al., 2009). Acute psychogenic (e.g. restraint) or systemic (e.g. hypoxia or visceral illness) stressors deplete PPG mRNA. Notably, PPG hnRNA is increased in the NTS, suggesting that stress increases PPG transcription (Zhang et al., 2009). Thus, the decrease in PPG mRNA is likely due to RNA degradation mediated by glucocorticoids acting in the NTS. Evidence for this hypothesis comes from studies finding that the effects of stress on PPG mRNA are attenuated in adrenalectomized rats and reproduced by exogenous corticosterone administration (Zhang et al., 2009). While acute stress briefly decreases PPG mRNA, both chronic variable stress and repeated restraint produce sustained down regulation (Ghosal et al., 2013; Zhang et al., 2010), an effect dependent on chronic stress-induced corticosterone secretion (Zhang et al., 2010). Collectively, these data suggest that glucocorticoids are crucial for limiting excess excitatory drive to the PVN through long-term down regulation of PPG mRNA. Additional evidence for glucocorticoid inhibition of HPA axis drive via the NTS come from pharmacological studies demonstrating the necessity and sufficiency of NTS GR signaling for decreasing HPA axis activity (Bechtold et al., 2009; Ghosal et al., 2014). In addition to these neuroendocrine effects, NTS GR is also required to diminish the impact of stress on anxiety- and depression-like behaviors (Ghosal et al., 2014). In aggregate, these studies

indicate that glucocorticoid-mediated mechanisms in the NTS are critical for moderating endocrine and behavioral stress responses and provide a basis for promoting organismal adaptation to chronic stress.

7.4. Glutamate

As previously mentioned, the excitatory neurotransmitter glutamate is expressed throughout the NTS, including noradrenergic and GLP-1 neurons (Niciu et al., 2012; Storm-Mathisen et al., 1983; Stornetta et al., 2002; Zheng et al., 2015). Thus, glutamate may serve as a major NTS synaptic input to PVN, with norepinephrine and GLP-1 acting as potent modulators (Fig. 2). Although NTS glutamatergic circuits are not well-characterized, within the PVN, glutamatergic synapses interact with diverse neuromodulatory systems to regulate HPA axis output (Bains et al., 2015). In fact, glutamatergic synapses often combine fast ionotropic signaling with slower-acting metabotropic signaling mediated by co-transmitters, including norepinephrine (Atasoy et al., 2012; Bains et al., 2015; Betley et al., 2013; Daftary et al., 2000). Importantly, HPA axis activity induced by electrical or pharmacological stimulation of the noradrenergic system is blocked by intra-PVN microinjection of glutamate receptor antagonists (Feldman and Weidenfeld, 2004). Furthermore, catecholaminergic inputs from the hindbrain promote glutamatergic excitation of parvocellular PVN neurons in response to glycemic challenge (Johnson and Watts, 2014). Postsynaptic glutamatergic currents in the PVN are also reduced by an adrenergic antagonist, suggesting that norepinephrine tonically enhances glutamate co-release in the PVN (Inoue et al., 2014). Taken together, these data indicate that glutamatergic innervation constitutes an important excitatory input to the PVN and substantially interacts with neuromodulatory systems such as norepinephrine and, perhaps, GLP-1 to regulate HPA axis dynamics.

8. Paraventricular hypothalamic integration of inputs

8.1. Synaptic plasticity

Brainstem input to the PVN is dynamically regulated by local circuit mechanisms within neuroendocrine cells. For example, CRH neurons exhibit stress-induced synaptic plasticity and also provide feedback to afferent synapses via retrograde signaling (for reviews see Bains et al., 2015; Herman et al., 2008; Hill and Tasker, 2012; Wamsteeker Cusulin and Bains, 2015). Briefly, chronic stress increases the expression of CRH (Herman et al., 1995) and ionotropic glutamate receptors (Herman et al., 2008), while reducing expression of GR (Herman et al., 1995) and GABA receptor subunits (Cullinan, 2000). Chronic stress also increases glutamatergic and noradrenergic appositions onto the somato-dendritic compartment of PVN CRH neurons (Flak et al., 2009). Interestingly, the chronic stress-induced increases in pre-synaptic input are abolished by PVN noradrenergic lesions (Flak et al., 2014), raising the possibility that plastic glutamate and norepinephrine afferents arise from the same cell population or that noradrenergic-glutamatergic interactions within the PVN are necessary for chronic stress-induced synaptic plasticity. In support of the later, norepinephrine promotes glutamate receptor-dependent synaptic potentiation following stress, allowing the PVN to undergo experience-dependent plasticity (Inoue et al., 2013). Collectively, the structural and functional consequences of chronic stress include up-regulation of neuronal processes promoting excitability, concomitant with decreased

capacity for negative feedback. Overall, this plasticity could account for facilitation of PVN output and enhanced HPA axis activity after chronic stress. However, CRH neurons have the capacity to release retrograde inhibitory signals that tune activity of afferent synapses (Di et al., 2003).

8.2. Retrograde signaling

Excitatory glutamatergic drive of CRH neurons is dampened by retrograde signaling, most prominently endocannabinoids and opioids (Evanson et al., 2010; Patel et al., 2004; Tasker et al., 2006; Wamsteeker Cusulin and Bains, 2015). Release of inhibitory messengers in the PVN is an activity-dependent process that activates pre-synaptic receptors and decreases the firing of afferents terminals (Di et al., 2009), including those from the brainstem. Specifically, CRH neuronal endocannabinoid release at glutamatergic synapses suppresses glutamate release and subsequent excitation of post-synaptic cells (Di et al., 2009). This process is dependent on cannabinoid receptor 1 (CB1) activation (Di et al., 2003), as well as GR (Nahar et al., 2015). Furthermore, CB1 antagonism reverses glucocorticoid feedback inhibition of the HPA axis (Evanson et al., 2010), indicating the necessity of CB1-GR interactions for terminating HPA axis response to stress. Importantly, repeated stress progressively decreases CB1 signaling, as well as GR feedback, resulting in the loss of endocannabinoid-mediated inhibition of PVN afferent neurotransmitter release (Wamsteeker et al., 2010). Thus, exposure to chronic or repeated stress disinhibits glutamatergic drive to parvocellular neurons, providing an additional avenue for sensitization of the HPA axis. More recently, an additional mechanism of retrograde signaling from neuroendocrine cells was described that relies on somato-dendritic release of endogenous opioids acting on pre-synaptic μ-opioid receptors (Wamsteeker Cusulin et al., 2013). In this case, prolonged activation of parvocellular neurons drives the release of opioids that lead to long-term synaptic depression in afferent terminals. Interestingly, this process is enhanced by GR and occurs at both glutamatergic and GABAergic synapses (Bains et al., 2015). This would seem to indicate that protracted HPA axis activity may result in a generalized μ-opioid-dependent inhibition of neurotransmitter release onto CRH neurons, thus reducing responsiveness to inputs from the brainstem.

9. Summary and conclusions

Monosynaptic inputs to PVN parvocellular neurosecretory cells arise from key brainstem nuclei, including the PAG, raphe nuclei, PBN, LC, and NTS. This closely associated group of structures provides diverse neurochemical input to the PVN, including glutamate, serotonin, norepinephrine, and a variety of neuropeptides. Neurons in these regions are activated by multiple modalities of stress and form a major HPA axis-regulatory network. This circuitry also underlies behavioral responses to stress, including, fear-, anxiety-, and depression-like behavior. The NTS appears to be particularly prominent for the integration of affective processes with physiological status, regulating many aspects of stress responding. Neurons of the NTS are also under regulatory control by glucocorticoid-mediated feedback, acting to constrain endocrine and behavioral responses to prolonged stress. At the level of the PVN, brainstem afferents are summated in an experience-dependent manner and modulated by post-synaptic retrograde messengers, providing a

highly-plastic environment. The responsiveness of PVN neurons to brainstem afferents is likely increased by chronic stress. In fact, chronic stress enhances glutamatergic signaling within the PVN, a process that requires norepinephrine and that may be facilitated by reduced endocannabinoid inhibition. In totality, the literature reviewed here suggests that the activity of specific brainstem neural circuits could account for many of the long-term deleterious effects of stress.

It is important to note that the current review focuses almost exclusively on literature generated from rodents. This is due to the vast quantity of data on pathways and neurochemical mechanisms that have emerged from rodent studies. Ultimately, advances in clinical outcomes will need to be driven by a better understanding of the parallel circuitry in humans. Currently, functional imaging modalities are limited in their spatial and temporal resolution, hampering the ability to discern the activity and interactions of individual brainstem nuclei. Nonetheless, there is evidence from human studies that brainstem nuclei are significant contributors to behavioral pathology. For instance, electrical stimulation of the PAG in humans produces fear and avoidance (Behbehani, 1995). Additionally, increased cerebrospinal fluid norepinephrine levels are observed in patients with melancholic depression (Wong et al., 2000), suggesting that brainstem noradrenergic neurons may contribute to the pathophysiology of depression.

The basic neurobiology of brainstem circuit integration also has several outstanding questions. There are a number of brainstem structures that have received little attention in relation to HPA axis regulation. For instance, the lateral dorsal tegmental nucleus and peduculopontine nucleus both give rise to cholinergic afferents that, although sparse, do innervate the PVN (Ruggiero et al., 1990; Ziegler and Herman, 2002). Additionally, the A1/C1 regions of the ventrolateral medulla provide norepinephrine/epinephrine input, respectively, to the PVN (Sawchenko and Swanson, 1982). These neurons mainly target magnocellular vasopressin neurons, although most techniques historically used to investigate noradrenergic signaling in the PVN have not clearly differentiated between the cell populations of origin. Furthermore, the complex physiology and functional consequences of co-transmitters and transmitter-peptide co-release have only recently begun to be unraveled. This interface is of paramount importance for a comprehensive understanding of circuit integration, as well as the mechanisms of stress-induced plasticity. These abundant interactions likely represent the endogenous signals guiding synaptic function, as well as endocrine and behavioral stress reactivity. Particularly, in-depth investigations of specific glutamatergic circuits and their co-transmitters could yield major advances in this realm. For instance, the role of PBN glutamate output for stress reactivity is poorly characterized, let alone the role of the numerous peptides likely co-released by these nuclei. Additionally, raphe serotonergic neurons have a mixed pattern of glutamate co-expression (Hioki et al., 2010), a phenomenon that has not been functionally investigated. Finally, the interface of GLP-1 and glutamate signaling within the PVN is unknown. Whether these mixed circuits and messengers interact synergistically or oppositionally is critical for delineating both basic neural physiology, as well as the complexity of neuropsychiatric disorders.

In conclusion, the growing consensus emerging from multiple lines of research on brainstem circuitry supports the idea that this region is vital for mediating many aspects of the

relationship between psychogenic stress and behavioral dysregulation. Furthermore, the previous theories that viewed the brainstem simply as a component of the 'reptilian' brain, only acting to stimulate reflexive responses to systemic disturbance, is no longer adequate for a thorough and coherent understanding of stress-regulatory neuronal networks. Moving forward, the interconnectedness and microcircuitry of brainstem nuclei, as well as the heterogeneity of synaptic chemistry, represent major frontiers for advancing stress neurobiology.

Acknowledgments

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Highlights

- Brainstem nuclei provide monosynaptic input to the paraventricular hypothalamus

- These distinct cell groups signal through multiple transmitters and peptides

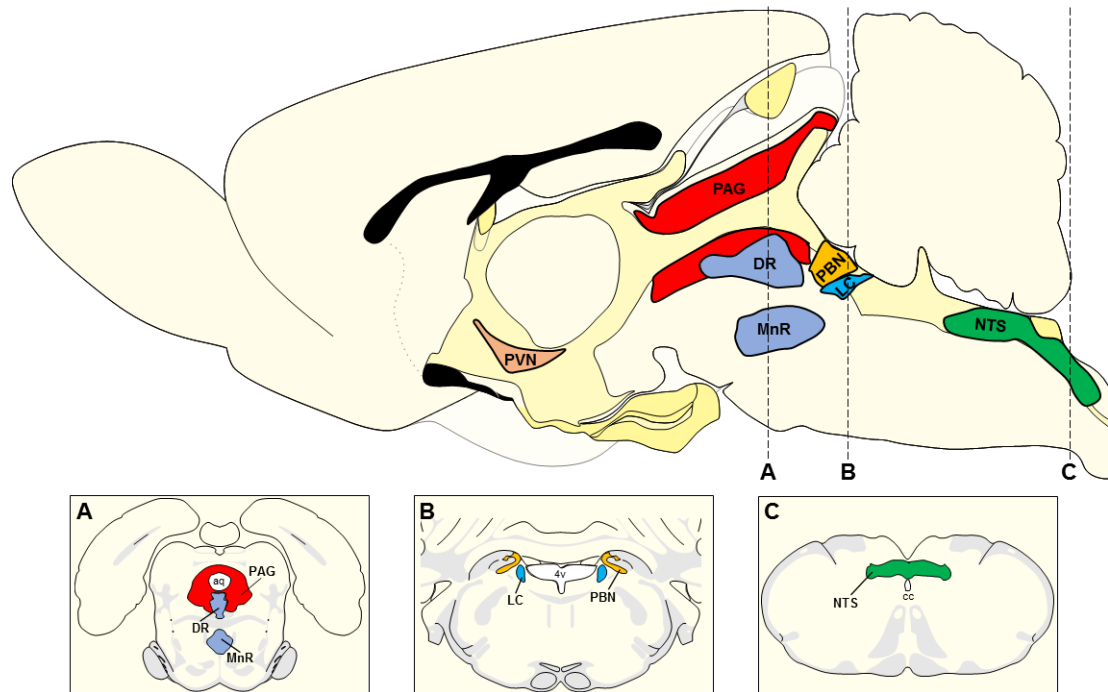- Integration of brainstem inputs is critical for appropriate stress responding

Figure 1. Anatomical diagram of PVN-projecting brainstem nuclei

Sagittal rat brain schematic adapted from Swanson 2004 indicating the relative location of major PVN-projecting brainstem regions. The *top panel* illustrates PVN (light brown), PAG (red), raphe nuclei (DR and MnR; light blue), PBN (orange), LC (cerulean blue), and NTS (green). *Panel A* depicts a coronal section through the midbrain as indicated by dashed line A. This coronal map adapted from Swanson 2004 indicates the location of the PAG, DR, and MnR relative to the aq. *Panel B* illustrates a coronal section corresponding to dashed line B and outlines the PBN and LC in relation to the 4v. *Panel C* is a schematic coronal section through the caudal medulla (dashed line C) depicting the location of the NTS relative to the cc. *List of abbreviations* aq: cerebral aqueduct, cc: central canal, DR: dorsal raphe, LC: locus coeruleus, MnR: median raphe, NTS: nucleus of the solitary tract, PAG: periaqueductal gray, PBN: parabrachial nuclei, PVN: paraventricular nucleus, 4v: fourth ventricle.

Input from Limbic Forebrain

(e.g. PFC + Amygdala)

NTS

Integrated Output

NE/Glu and GLP-1/Glu

Anxiety + Depression-like Behavior

HPA Axis Activation

Sympatho-excitation

Interoceptive Information

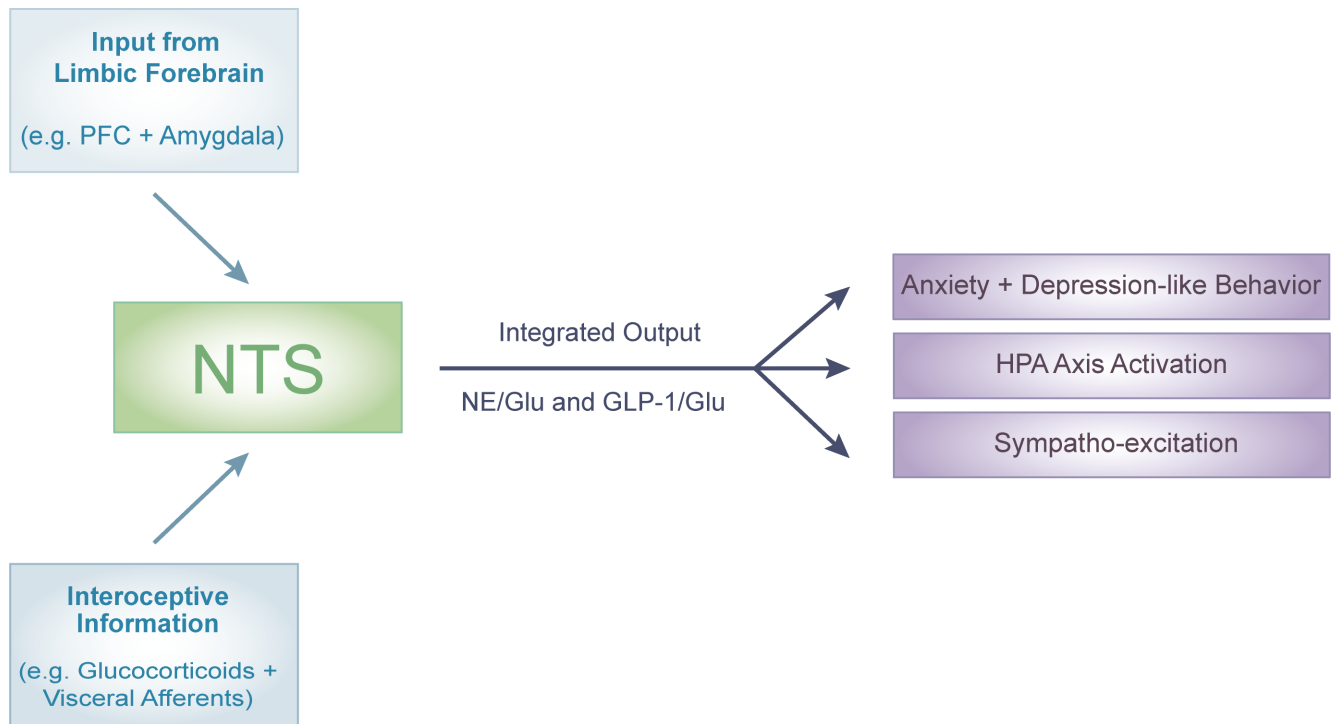(e.g. Glucocorticoids + Visceral Afferents)

Figure 2. Summary of NTS as nexus for integrating descending limbic input with ascending interoceptive information to generate behavioral, neuroendocrine, and autonomic stress responses

Regions of the limbic forebrain including the PFC and amygdala, among others, project to the NTS. The NTS also expresses a high density of glucocorticoid receptors and receives ascending visceral signals related to physiological status. At the level of the NTS, this information is integrated and multiple output circuits including NE/Glu and GLP-1/Glu coordinate organismal adaptation to adversity. *List of abbreviations* GLP-1: glucagon-like peptide-1, Glu: glutamate, NE: norepinephrine, NTS: nucleus of the solitary tract, PFC: prefrontal cortex,