Hindbrain noradrenergic A2 neurons: diverse roles in autonomic, endocrine, cognitive, and behavioral functions

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Rinaman L. Hindbrain noradrenergic A2 neurons: diverse roles in autonomic, endocrine, cognitive, and behavioral functions. *Am J Physiol Regul Integr Comp Physiol* 300: R222–R235, 2011. First published October 20, 2010; doi:10.1152/ajpregu.00556.2010.—Central noradrenergic (NA) signaling is broadly implicated in behavioral and physiological processes related to attention, arousal, motivation, learning and memory, and homeostasis. This review focuses on the A2 cell group of NA neurons, located within the hindbrain dorsal vagal complex (DVC). The intra-DVC location of A2 neurons supports their role in vagal sensory-motor reflex arcs and visceral motor outflow. A2 neurons also are reciprocally connected with multiple brain stem, hypothalamic, and limbic forebrain regions. The extra-DVC connections of A2 neurons provide a route through which emotional and cognitive events can modulate visceral motor outflow and also a route through which interoceptive feedback from the body can impact hypothalamic functions as well as emotional and cognitive processing. This review considers some of the hallmark anatomical and chemical features of A2 neurons, followed by presentation of evidence supporting a role for A2 neurons in modulating food intake, affective behavior, behavioral and physiological stress responses, emotional learning, and drug dependence. Increased knowledge about the organization and function of the A2 cell group and the neural circuits in which A2 neurons participate should contribute to a better understanding of how the brain orchestrates adaptive responses to the various threats and opportunities of life and should further reveal the central underpinnings of stress-related physiological and emotional dysregulation.

catecholaminergic; dorsal vagal complex; nucleus of the solitary tract; stress; visceral

THE MAMMALIAN BRAIN STEM CONTAINS several distinct groups of noradrenergic (NA) neurons that were initially described by Dahlström and Fuxe (59) who labeled the groups A1 through A7 as they extend from the caudal ventrolateral medulla through the rostral lateral pons. NA neurons collectively project throughout the central nervous system and are broadly implicated in behavioral and physiological processes related to attention, arousal, motivation, learning and memory, and homeostasis. NA neurons are distinguished by positive immunolabeling for tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis, and dopamine-β-hydroxylase (DbH), the enzyme that converts dopamine to norepinephrine (NE) (8). Conversely, neurons comprising the A1-A7 cell groups are not immunopositive for phenylethanolamine N-methyltransferase (PNMT). PNMT catalyzes the synthesis of epinephrine from NE, and its presence is used to identify adrenergic neurons of the C1-C3 cell groups (59).

This review focuses on the A2 cell group, a fascinating collection of NA neurons contained within the dorsal vagal complex (DVC) in the caudal dorsomedial medulla (see Fig. 1). As discussed further, below, the intra-DVC location of A2 neurons supports their known involvement in vagal sensory-motor reflex arcs and vagal motor outflow to multiple visceral targets. Perhaps less well appreciated is the role of A2 neurons in processes as diverse as satiation, sickness behavior, affective state, endocrine and behavioral stress responses, immune-to-brain signaling, emotional learning, memory consolidation, and addictive drug dependence. A2 neurons participate in reciprocal connections between the visceral DVC and other medullary, pontine, diencephalic, and telencephalic brain regions that underlie these diverse processes. Direct projections from the cortex, limbic forebrain, and hypothalamus to the region of the A2 cell group provide a route through which emotional and cognitive events can modulate visceral responses to diverse threats and opportunities to which the organism is exposed, including conditioned responses that are based on past experience (193, 225). In turn, ascending projections from A2 neurons provide a route through which interoceptive feedback from the body impacts not only hypothalamic functions but also emotional and cognitive processing (21, 141, 211, 225).

The neuroanatomical and phenotypic features of A2 neurons will first be considered in this review, followed by a summary of evidence that A2 neurons provide a critical brain-body interface linking emotional/cognitive events with physiological effects.
support, especially during stressful events that challenge bodily homeostasis. This general theme will be supported by briefly reviewing the involvement of A2 neurons in food intake, affective behavior, stress responses, emotional learning, and drug dependence. Most of the information reviewed in this article was derived from studies using rats and, to a lesser extent, mice; however, central NA circuits are highly conserved across mammalian species. Thus, understanding the functional organization of the A2 cell group in rodents has clinical relevance, and should contribute to a better understanding of stress-related physiological and emotional dysregulation in humans.

### Anatomical and Neurochemical Features

#### Location of A2 neurons.

The A2 cell group is centered within intermediate and caudal levels of the nucleus of the solitary tract (NST) (Fig. 1), referred to as the visceral NST to distinguish these levels from the more rostral gustatory NST (143). The visceral NST is a key component of the DVC, which also includes the area postrema (AP) and dorsal motor nucleus of the vagus. The DVC is a critical central node for controlling hormonal and autonomic outflow, and relaying interoceptive feedback from body to brain (201, 205, 206, 291). The AP and a significant portion of the medial NST underlying the AP contain fenestrated capillaries, allowing blood-borne factors (e.g., hormones, toxins, cytokines) to affect A2 and other neurons local to this region. Within the DVC, AP neurons innervate the subjacent NST, and NST neurons innervate other NST neurons as well as vagal preganglionic parasympathetic neurons whose cell bodies occupy the dorsal motor nucleus of the vagus and whose dendrites ramify widely within the NST (239). All three components of the DVC also receive extrinsic neural inputs from the periphery and brain, described further, below.

Although the A2 cell group is centered within the visceral NST, A2 neurons are not confined to cytoarchitecturally-distinct NST subnuclei. Instead, they form two bilaterally symmetrical loose linear columns of medium-sized ovoid or multipolar cells that extend rostrocaudally through the visceral NST (232) (Fig. 1). A2 neurons are most prevalent within the medial subnucleus of the NST at the rostrocaudal level of the AP, but they also exist within the NST commissural subnucleus at the level of the AP and more caudally. Furthermore, some A2 neurons are located within and just lateral to the cytoarchitectural boundaries of the dorsal motor nucleus of the vagus (232). The most caudal A2 neurons are located in the upper cervical spinal cord, and the most rostral are located rostral to the AP at the level of the caudal fourth ventricle (Fig. 1). It should here be noted that the more rostral A2 neurons are intermixed with PNMT-positive adrenergic neurons of the C2 cell group (Figs. 1 and 2), which also are TH- and DbH-positive. Many of the neuroanatomical and functional studies cited in this review relied on anatomical localization together with TH or DbH immunolabeling to...
identify and/or lesion A2 neurons; however, these criteria do not allow A2 and C2 neurons to be distinguished within visceral NST regions where they overlap. The extent to which the connections and functions of these rostral A2/caudal C2 neurons are similar or unique remains largely unexplored.

**Transmitter coexpression by A2 neurons.** When considering the functional role of A2 projection systems, it’s important to keep in mind that these NA neurons release more than just NE from their axon terminals and varicosities. In addition to TH and DbH, A2 neurons express mRNA and/or are immunopositive for many additional signaling molecules. In rats, ~80% of A2 neurons reportedly express mRNA for a homolog of the vesicular glutamate transporter-2 (248), suggesting that these neurons release glutamate along with NE. In addition, virtually all A2 neurons are immunoreactive for prolactin-releasing peptide (PrRP) (52). PrRP receptors are expressed within the paraventricular nucleus of the hypothalamus (PVN) and other brain regions targeted by A2 neurons (284), and there is evidence that PrRP acts synergistically with NE to activate hypophysiotropic corticotropin-releasing hormone (CRH) neurons at the apex of the hypothalamic-pituitary-adrenal (HPA) axis (147, 261). Interestingly, the ratios of PrRP to NA biosynthetic enzymes in A2 neurons are modulated by estrogen and stress (235), and A2 neurons express receptors for estrogen and glucocorticoids (56, 101, 198, 243).

Regarding other peptides and phenotypic markers, subpopulations of A2 neurons are immunopositive for neuropeptide Y (83, 233), nesfatin-1 (23), dynorphin (40), neurotensin (200), and/or pituitary adenylate cyclase-activating polypeptide (61). Conversely, A2 neurons apparently do not colocalize galanin (136), somatostatin (226, 227), enkephalin (226), inhibin-β (226), glucagon-like peptide 1 (133, 203), or the enzyme 11β-hydroxysteroid dehydrogenase-2 (99), despite their close anatomical proximity to NST neurons, which do express these various phenotypic markers. A2 neurons also do not appear to coexpress cocaine and amphetamine-related transcript, neotensin, or cholecystokinin, in contrast to coexpression of these peptides by PNMT-positive neurons of the partially overlapping C2 cell group (84, 122).

**Receptor expression by A2 neurons.** Neurons within the A2 region of the visceral NST express receptor mRNA and binding sites for a large number of neurotransmitters and other signaling molecules, although confirmation of receptor expression by identified A2 neurons is relatively limited. The available data indicate that A2 neurons express α2A adrenergic receptors (154, 221) as well as receptors for glutamate (6, 89), GABA (116), cannabinoids (38), CRH (171), neuropeptide Y (288), leptin (80), glucocorticoids (219), and estrogen (56, 198, 243). A2 neurons do not express mineralocorticoid receptors (99).

**Extrinsic Inputs and Axonal Projections**

**Sensory inputs to A2 neurons.** In addition to local axonal inputs from the superjacent AP that are positioned to relay blood-borne signals to A2 neurons (54, 118, 238), the A2 region of the visceral NST receives sensory feedback from the cardiovascular, respiratory, and alimentary systems (119). These visceral sensory inputs arrive predominantly via glutamatergic glosopharyngeal and vagal afferents whose central axons converge in the solitary tract before synapsing with the dendrites and somata of NST and vagal motor neurons (5, 14, 208, 246). In mice, ~90% of A2 neurons receive direct synaptic input from visceral afferents in the solitary tract (6). These glutamatergic inputs produce tightly synced, large-amplitude excitatory postsynaptic currents in A2 neurons, providing high-fidelity transmission of sensory afferent activity (6). Other visceral and somatic sensory inputs are relayed to the A2 region of the NST from the spinal cord, trigeminal and related nuclei, and reticular formation (5, 7, 71, 152, 153).

Given the diversity of sensory inputs received by A2 neurons, it is not surprising that they respond to a broad array of interoceptive signals, including hormonal, osmotic, gastrointestinal, cardiovascular, respiratory, and inflammatory signals (20, 23, 43, 45, 66, 77, 97, 112, 120, 167, 168, 201, 202, 207, 212, 213, 230). In these and many other studies, stimulus-induced A2 neuronal activation is characterized by immunocytochemical localization of the immediate-early gene product, Fos, together with immunolabeling for TH or DbH. Increased Fos immunolabeling alone cannot reveal the circuits through which A2 neurons are recruited by a given stimulus or event, but A2 neurons are consistently activated by treatments or situations that present actual or anticipated threats to bodily homeostasis. In many cases the relevant information is communicated to A2 neurons by visceral sensory afferents, but in other cases A2 neurons appear to be recruited by descending inputs from the hypothalamus and limbic forebrain (30, 67, 68, 137, 138). These inputs are reviewed in the following section.

**Central inputs to A2 neurons.** Retrograde and anterograde tract-tracing studies have revealed a wide array of brain stem and forebrain nuclei that project directly to the visceral NST and may participate in recruitment of A2 neurons. As summarized in Table 1, these include various regions of the medullary, pontine, and mesencephalic reticular formation (13, 157, 166, 180); the cerebellar fastigial nucleus (180); the raphé obscurus, pallidus, magnus, paragigantocellularis, and parapyramidal region (144, 180, 256); the retrotrapezoid nucleus (220); the parabrachial nucleus and Kölliker-Fuse nucleus (130, 180); the periaqueductal gray (180); the hypothalamic tuberomammillary nucleus (182); the hypothalamic arcuate nucleus (266, 295); the PVN (100, 204, 228, 245, 266); the lateral hypothalamic area (180, 266, 294); the median preoptic nucleus (180); the dorsomedial hypothalamus (287); the central nucleus of the amygdala (CeA) and anterolateral bed nucleus of the stria terminalis (aBST) (116, 140, 180, 222, 266); the lateral septal nucleus (180); and glutamatergic pyramidal neurons in the insular cortex and in prelimbic and infralimbic regions of the medial prefrontal cortex (266). All of these brain regions are logical candidates for sources of direct input to A2 neurons, although dual-labeling electron microscopy is necessary to confirm synaptic connectivity. The limited ultrastructural evidence that is available indicates that at least a subset of A2 neurons receive synaptic input from noncatecholaminergic neurons in the adjacent AP (118), and at least some A2 neurons receive glutamatergic inputs from the infralimbic region of the medial prefrontal cortex (94). It also seems likely that A2 neurons are among the catecholaminergic neurons within the visceral NST that receive synaptic input from the CeA (189) and from orexin-positive neurons in the lateral hypothalamus (17).

**Axonal projections of A2 neurons.** A2 neurons project locally within the DVC and medullary reticular formation, and...
Table 1. Central connections of the A2 region of the dorsal vagal complex

<table>
<thead>
<tr>
<th>Central Connections</th>
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<tbody>
<tr>
<td>• Spinal cord, cranial nerves</td>
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<td>• Dorsal horn and lamina X</td>
</tr>
<tr>
<td>• Glossopharyngeal and vagal sensory afferents (via solitary tract)</td>
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<tr>
<td>• Medulla, Pons, and Midbrain</td>
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<tr>
<td>• Dorsal motor nucleus of the vagus</td>
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<tr>
<td>• Nucleus ambiguous</td>
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<tr>
<td>• Area postrema</td>
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<tr>
<td>• Trigeminal and related nuclei</td>
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<tr>
<td>• Medullary, pontine, mesencephalic reticular formation</td>
</tr>
<tr>
<td>• Raphe obscurus, pallidus, magnus, paragigantocellularis, and parapyramidal region</td>
</tr>
<tr>
<td>• Locus coeruleus and peri-locus coeruleus region</td>
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Cerebellar fastigial nucleus
Parabrachial nucleus
Kölliker-Fuse nucleus
Lateralodorsal tegmental nucleus
Ventral tegmental area
Retrotrapezoid nucleus
Periaqueductal gray
Thalamus and hypothalamus
Midline thalamic nuclei
Tuberomammillary nucleus
Arcuate nucleus
Paraventricular nucleus
Lateral hypothalamic area
Dorsomedial nucleus
Median preoptic nucleus
Supraoptic nucleus
Subfornical organ
Telencephalon
Central nucleus of the amygdala
Anterolateral bed nucleus of the stria terminals
Substantia innominata
Nucleus accumbens
Lateral septal nucleus
Insular cortex
Medial prefrontal cortex

Italicized = source of axonal input to the A2 region; Underlined = target of A2 axonal projections; Bold = both a source of axonal input to the A2 region and a target of A2 axonal projections. Citations are provided in text.

A subset of individual A2 neurons have axon collaterals that innervate both the PVN and supraoptic nuclei (195, 196, 232). In the absence of non-NA projections from the NST to the caudal ventrolateral medulla (111), it is relevant to note here that A2 neurons may release their transmitter synaptically and in a paracrine manner, requiring that NE and other costored transmitters must diffuse short distances through the extracellular space to bind to cognate receptors (Ref. 190).

Fig. 3. Dual immunofluorescence labeling of anterogradely-transported Phaseolus vulgaris leucoagglutinin (PhAL) neural tracer (green) and DbH (red) identifying axons in the medial parvocellular (mp) and lateral magnocellular (lm) subregions of the paraventricular nucleus of the hypothalamus (PVN). PhAL was microinjected iontophoretically into a portion of the A2 region in an adult male Sprague-Dawley rat 14 days before death (see Ref. 201).
likely shared by the other. The following sections review several examples of functions in which A2 neurons have been implicated, but the reader should consider that A1 neurons also are likely to be involved in at least a subset of these functions.

**A2 Neurons and Food Intake**

Central NA signaling pathways, including those that arise from A2 neurons, appear to be essential for inhibiting or stimulating food intake under different conditions (2, 62, 142, 156, 170, 203, 209, 214, 216). It seems likely that different subpopulations of A2 neurons with distinct axonal projections are recruited by signals that increase or decrease food intake, perhaps because different subpopulations target different brain regions, and/or because different combinations of adrenergic receptors are expressed within those regions (277). Interestingly, A2 neurons in rats appear to be activated in every experimental situation in which food intake is inhibited, including normal satiety (37, 115, 203, 207, 262). A2 neurons are recruited in a graded manner in rats after voluntary food intake, such that larger meals activate larger numbers of A2 neurons (207). Not only are A2 neurons robustly activated in rats after systemic administration of cholecystokinin octapeptide (210, 213) (Fig. 4), they are necessary for the ability of cholecystokinin to inhibit food intake and to activate neurons within the hypothalamic PVN (203). A2 neurons also contribute importantly to the hypophagic effect of lithium chloride (209). Food intake is reduced in rats after central administration of PrRP (134) or nesfatin-1 (174), each of which is coexpressed by A2 neurons (23, 52). A2 neurons are robustly activated by experimental treatments or situations that produce hypophagia or anorexia as a part of the depressive-like sickness behavior accompanying systemic infection or visceral malaise (23, 96–98, 201, 202). These conditions also are associated with inhibition of vagally mediated gastric emptying, which likely underlies or contributes to hypophagia (37, 202, 203). The potential involvement of A2 neurons in other aspects of food intake and regulation of body energy homeostasis are the subject of a recent review (201).

**Fig. 4.** Dual immunoperoxidase labeling of cFos protein (blue/black nuclear label) and cytoplasmic DbH (brown label) within the caudal visceral NST. This section was taken from a rat perfused with fixative 60 min after intraperitoneal administration of cholecystokinin octapeptide (100 μg/kg body wt) to stimulate vagal sensory inputs to the NST. Arrows point out activated (i.e., cFos-positive) A2 neurons (see Refs. 210 and 213).

**A2 Neurons, the LC, and Affective Behavior**

The trajectory and targets of NA fibers within the VNAB are distinct from those of the dorsal NA bundle, which originates from neurons within the pontine LC (A4 and A6 cell group regions). The vast majority of publications considering the role of central NA signaling in stress, cognition, and affective processes emphasize the LC and its projection targets, and either disregard or downplay the contributions of A2 neurons and their projections. The LC is assumed to be the principle source of the central NA signaling that underlies not only behavioral arousal but also HPA axis hyperactivity associated with stress (48–50, 289) as well as the dysregulated NA transmission that contributes to diverse models of stress vulnerability and affective disorders (9, 51). However, NA inputs to the PVN, CeA, aBST, and NAcc that are critical for hormonal, behavioral, and affective responses to physiologically significant events arise from the caudal medullary A1 and A2 cell groups, with relatively little input from the LC (70, 230, 232). When clinical researchers measure growth hormone responses to an adrenergic agent (e.g., clonidine) to indirectly assess brain NA signaling in individuals with stress-related affective disorders (1, 72, 241, 242), it is primarily NA signaling by medullary A1/A2 inputs to the hypothalamus that is being assessed (47, 81, 165, 282), not LC inputs. Furthermore, results from a recent retrograde tracing and VNAB lesion study indicate that the majority of NA inputs to reward-related midbrain dopamine neurons arise from the caudal medulla, including the A2 cell group (151). Conversely, A2 neurons do not innervate most of the brain regions that are innervated by the LC, including the olfactory bulb, cerebral cortex, hippocampus, medial BST, basolateral amygdala, most thalamic nuclei, and the cerebellum (163). Moreover, NA signaling within LC-innervated brain regions can produce behavioral effects that are quite different from those produced by NA signaling within A2-innervated regions. For example, α-adrenergic receptor activation underlies positively motivated exploratory/approach behavior in cortical and subcortical regions innervated by the LC, but underlies stress reactions with behavioral inhibition in regions innervated by the A2 cell group (29, 41, 42, 69, 164, 192, 215, 247, 277, 278).

Neurons within the A2 region of the visceral NST project to the LC (201), but project more densely to the peri-LC region where the dendrites of LC neurons cluster, synapsing there on TH-positive dendrites (264, 265). As mentioned previously, A2 neurons coexpress PrRP immunolabeling (52), and the LC contains PrRP-positive fibers and terminals as well as the receptor (UHR-1) for PrRP (284). While the evidence is indirect, these findings indicate that A2 neurons may provide modulatory control over NA neurons within the LC, thereby indirectly modifying NA signaling within cortical, hippocampal, amygdalar, thalamic, and cerebellar targets of the LC. Interestingly, despite the LC-centered focus of most research on the role of NA brain systems in affective behavior, researchers have not been able to demonstrate unequivocally the necessity of LC neurons in fear, anxiety, or depressive-like behavior (114). Indeed, LC lesions appear to increase, rather than decrease, novelty-induced fear and anxiety in rats (103, 148). Moreover, in one study, LC lesions increased the antidepressant-like effect of reboxetine (a NE reuptake inhibitor), whereas VNAB lesions abolished the drug’s antidepressant
effects (53). These results invite a continued expansion of research into the role of A2 neurons and their central projections in affective behavior.

**A2 Neurons and Stress Responses**

Stressors are stimuli or events that challenge (or are perceived to challenge) bodily homeostasis and well-being. Signals generated by stressors may initially arise from the environment (e.g., visual or olfactory signals), or may arise from within the body (e.g., cardiovascular or gastrointestinal signals). Physiological and behavioral responses to stressful stimuli are the product of interactions among multiple brain regions (107, 108). However, three interconnected regions of the hypothalamus and so-called extended amygdala are especially important, and have been the subject of extensive experimental attention: the medial parvocellular PVN (mpPVN), CeA, and alBST. Each region contains CRH neurons that receive synaptic input from NA terminals arising primarily or exclusively from the A2 (and A1) cell groups, and CRH neuronal activity within each region is closely regulated by these inputs (4, 10, 65, 74, 78, 79, 124, 126–128, 139, 181, 186, 191, 192, 244). A2 neurons express glucocorticoid receptors (101), and central adrenergic and CRH receptors are regulated by glucocorticoids, which are known to affect NE synthesis and turnover throughout the brain. NA-CRH signaling pathways are viewed as part of a central adrenal steroid-sensitive network that tunes physiological and behavioral responses during conditions of acute or chronic stress (106). In general, and as discussed further, below, NA signaling is pivotal in regulating HPA and behavioral responses to stress, and can modulate unconditioned and conditioned behavioral responses to stressful and emotional stimuli, including stimuli that evoke fear and anxiety (223). Enhanced NA transmission in human subjects is associated with enhanced HPA axis responses to stress, which may contribute to the psychopathology of depression, anxiety, and other affective disorders (128). The hindbrain A2 cell group appears to be a fundamental player in these central mechanisms, as summarized in the following sections.

**PVN.** Most hypothalamic NA input arrives from medullary NA cell groups. A2 neurons appear to selectively target the mpPVN (55, 230, 232), although axonal projections from the A2 region to the lateral magnocellular PVN also are common (see Fig. 3 and discussion in the following section). The necessity of NA inputs for HPA axis responses to stress appears to vary across different types of stress stimuli (20, 66, 137, 224, 229), but NA input to the mpPVN, arriving via the VNAB, provides the major known stimulus for CRH synthesis and release (65, 181, 192, 250, 283). CRH is the principal and obligate hypophysiotropic peptide driving the pituitary-adrenal axis under basal conditions and in response to homeostatic challenge (192, 274).

In rats, stressful stimuli that activate A2 neurons and recruit the HPA axis also activate hypothalamic oxytocin neurons (177, 178, 206, 263, 267–269). CRH and oxytocin neurons receive direct synaptic input from NA terminals, and NA inputs increase CRH and oxytocin excitability (3, 4, 22, 113, 155, 192, 195, 196, 217, 285). In late pregnancy and during lactation, oxytocin and HPA axis responses to stressors are attenuated by mechanisms that reduce NA tone within the PVN (26–28, 76, 257, 258). Lesions that decrease NA input to the mpPVN markedly attenuate CRH neuronal responses to interoceptive signals (20, 90, 137, 203, 209, 215, 234). Conversely, chronic stress sensitizes HPA axis responses to central NA (183) and increases the density of glutamatergic and NA synaptic inputs to CRH-positive neurons, evidence for enhanced signaling capacity (86). The authors of the latter study did not consider whether the increased glutamatergic and NA inputs arise from the same A2 neurons, but this seems likely.

Magnocellular oxytocin neurons and parvocellular thyrotropin releasing hormone-positive mpPVN neurons also receive synaptic input from the A2 cell group (57, 58, 93, 240, 283). In addition, nonendocrine gastric preautonomic neurons in the PVN receive direct synaptic input from NA nerve terminals that include inputs from A2 neurons (15). Preautonomic PVN neurons project to hindbrain and spinal centers to control autonomic motor outflow to the gastrointestinal tract and other organ systems (16, 87, 204, 251) to thereby shape visceral responses to emotive stimuli and stress (228, 292).

**alBST.** The anterolateral group of BST nuclei (alBST) includes the juxtacapsular, oval, rhomboid, fusiform, and subcommissural zone (75). The alBST is connected with autonomic-related portions of the hypothalamus and caudal medulla, and receives an extremely dense NA innervation that arises from A2 (and A1) neurons, but not from the LC (11, 25, 69, 74, 75, 186–188, 252, 275). NA acts within the alBST to modulate behavioral, hormonal, and conditioned emotional responses to stress (41, 179), including, for example, responses to the stress of precipitated opiate withdrawal (11, 78). The alBST also receives input from the hippocampus and prefrontal cortex, and has abundant projections to the mpPVN (74). At least a subset of A2 neurons that innervate the alBST have axon collaterals that target the mpPVN (16, 20), and NA signaling within the alBST contributes to stress-induced HPA axis activation (88, 135). Certain aspects of BST-mediated anxiety responses appear to depend on CRH inputs from the amygdala (63, 270, 271) with which the alBST is strongly and reciprocally connected (73). Blockade of NA signaling in the ventral alBST reduces immobilization stress-induced anxiety in the elevated-plus maze and attenuates immobilization stress-induced increases in plasma ACTH, but the same pharmacological manipulation has no effect to attenuate stress responses in a subsequent social interaction test (41); the reverse is true for similar manipulations in the CeA (42). These findings suggest that A2 inputs to the alBST and CeA are involved in different specific components of stress and anxiety responses (cf. Ref. 244).

**CeA.** The CeA, like the alBST, is a subcortical limbic structure characterized by its extensive connections with the hypothalamus and with brain stem viscerosensory and autonomic control nuclei. As part of the striatal amygdala, CeA neurons are primarily GABAergic and coexpress CRH, similar to neurons in the alBST (73, 249). Although NA inputs to the CeA are significantly less dense than NA inputs to the mpPVN and alBST (16, 167, 169), NA signaling within the CeA modulates behavioral responses to stressful events, including fear, anxiety, and avoidance behavior (64, 125). NA signaling in the CeA increases during precipitated opiate withdrawal and contributes to the negative affective (i.e., aversive) consequences of withdrawal (273), similar to increased NA signaling and implication in aversive effects within the alBST (11). A2 neurons that project to the CeA are activated in rats after
gastric vagal sensory stimulation with exogenous cholecystokinin (169) or systemic immune challenge (97), and also are activated by emotionally salient exteroceptive stimuli, such as exposure to a predator odor (168).

A2 Neurons and Emotional Learning

The effectiveness with which emotionally significant experiences are encoded into long-term memory is dependent, at least in part, on interoceptive feedback from body to brain, and increased NA signaling within the limbic forebrain is strongly implicated in emotional learning (31–35, 85, 161, 162, 279, 280). As reviewed earlier in this article, interoceptive signals funneled through the hindbrain DVC engage A2 neurons, including those that project to the amygdala and NAcc. Both limbic regions play a crucial role in the encoding, storage, and retrieval of memories associated with emotionally significant events. Activation of NA receptors within the amygdala and NAcc influences synaptic changes that are necessary at the time of encoding to facilitate long-term memory for emotional events (85, 124). NE release in the amygdala has been established as a neural substrate for memory modulation elicited by peripheral arousal (85, 280, 281), and NA inputs to the NAcc contribute importantly to the processing of appetitive and aversive reinforcement signals that impact learning and memory (70). Neurons within the amygdala and NAcc respond to gastric and cardiovascular vagal sensory signals that engage the A2 cell group (126, 150, 159). These A2 inputs play a key role in modulating amygdalar and NAcc activity by releasing NA in response to heightened states of arousal, providing a clear anatomical route through which emotional (i.e., visceral) signals can modulate learning and memory (124).

A2 inputs to other brain regions may affect learning and memory in a more indirect manner. For example, A2 inputs to NA neurons in the LC (264, 265) and to dopamine neurons in the midbrain retrorubral field (151) that innervate the entorhinal cortex and hippocampus may contribute to the modulation of declarative and spatial memory processes.

A2 Neurons in Drug Use and Dependence

Central neural adaptations elicited by exposure to addictive drugs are not limited to brain reward circuits, but also are manifest in stress-related pathways that are implicated in addiction (173). For example, rats dependent on morphine display increased enzymatic activity of A2 neurons and increased NA turnover within the PVN, concurrent with enhanced activity of the HPA axis that depends on NA input (18, 92, 131, 132, 172). In addition, medullary NA inputs to the alBST, CeA, and NAcc are critical for the aversiveness of acute opiate withdrawal, and for stress-induced relapse of drug seeking for opiates, cocaine, ethanol, and nicotine (11, 24, 36, 69, 78, 145, 244, 293). NA inputs to the alBST trigger GABAergic inhibition of alBST neurons that project to the ventral tegmental area, which likely contributes to the inhibition of DA neurons that occurs during opiate withdrawal (78). Thus, common inputs to the hypothalamus and limbic forebrain from the A2 cell group could be a critical factor linking these brain areas in circuits that underlie drug use and dependence (102, 236, 237).

Even 5 wk after opiate withdrawal, neurons within these limbic forebrain regions remain hypersensitive to drug-related cues and stress, which may drive behavior away from the pursuit of natural rewards, such as food and sex, and toward drug-related rewards to thereby perpetuate a cycle of drug addiction (102). Clinical observations of former opiate addicts revealed a prolonged hyper-responsiveness to stress, including altered cortisol release (129), which may be at least partly due to altered function of A2 signaling pathways. For example, A2-to-NAcc projection neurons are similarly activated by noxious visceral stimuli and by precipitated opiate withdrawal (69, 117), and the same projection pathway is implicated in cannabinoid modulation of NAcc activity and cannabinoid-induced aversion (38, 39).

Increased NA release within the extended amygdala continues to influence stress and anxiety systems in the brain for some time following acute drug withdrawal, even after somatic signs dissipate (91). Interestingly, conditioned preference for morphine is absent in DbH knock-out mice in which NE synthesis is interrupted, but preference is restored if DBH is rescued to restore NA signaling within and from the NTS, but not the LC (176). Repeated nicotine self-administration increases NA receptor sensitivity in the PVN and also enhances HPA function (290). Collectively, it seems that NA signaling from the A2 cell group to the hypothalamus and limbic forebrain contributes to mechanisms that support drug seeking and self-administration, increased anxiety during drug abstinence, altered reward processing (i.e., dysphoria), and the general relationship between the use of mood-altering drugs and mood disorders (12, 38, 78, 131, 176, 244).

Conclusion

The diverse challenges and opportunities of life elicit a constellation of autonomic, endocrine, cognitive, and behavioral responses, and A2 neurons are poised to contribute to the central coordination and modulation of these responses. As reviewed in this article, feedback regarding the body’s physiological state is relayed by A2 neurons to multiple regions of the brain stem, hypothalamus, and limbic forebrain. The A2 cell group is best defined by its afferent and efferent connections within a complex neural network that extends from the spinal cord to the cortex. The available evidence indicates that by virtue of their central axonal projections, this relatively small group of caudal medullary neurons can modulate ongoing and future physiological processes and behaviors, and may also contribute to the affective, contextual, and cognitive attributes of experience that depend on interoceptive feedback from body to brain (19, 60, 149, 194, 255).

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DISCLOSURES

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REFERENCES


Laorden ML, Núñez C, Almela P, Milanés MV. Morphine withdrawal-induced c-fos expression in the hypothalamic paraventricular nucleus is...


Saha S, Batten TFC, Henderson Z. A GABAergic projection from the central nucleus of the amygdala to the nucleus of the solitary tract: a


262. Van Boeckel AE, Peoples J, Telegen P. Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain:


