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Ascending projections from the caudal visceral nucleus of the solitary tract to brain regions involved in food intake and energy expenditure

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Abstract

Metabolic homeostasis reflects the complex output of endocrine, autonomic, and behavioral control circuits that extend throughout the central nervous system. Brain regions that control food intake and energy expenditure are privy to continuous visceral sensory feedback signals that presumably modulate appetite, satiety, digestion, and metabolism. Sensory signals from the gastrointestinal tract and associated digestive viscera are delivered to the brain primarily by vagal afferents that terminate centrally within the caudal nucleus of the solitary tract (NST), with signals subsequently relayed to higher brain regions by parallel noradrenergic and peptidergic projection pathways arising within the NST. This article begins with an overview of these ascending pathways identified in adult rats using a standard anterograde tracer microinjected into the caudal visceral sensory region of the NST, and also by immunocytochemical localization of glucagon-like peptide-1. NST projection targets identified by these two approaches are compared to the distribution of neurons that become infected after inoculating the ventral stomach wall with a neurotropic virus that transneuronally infects synaptically-linked chains of neurons in the anterograde (i.e., ascending sensory) direction. Although the focus of this article is the anatomical organization of axonal projections from the caudal visceral NST to the hypothalamus and limbic forebrain, discussion is included regarding the hypothesized role of these projections in modulating behavioral arousal and coordinating endocrine and behavioral (i.e., hypophagic) responses to stress.

Keywords

glucagon-like peptide-1; noradrenergic; hypothalamus; phaseolus vulgaris leucoagglutinin; transneuronal viral tracing

1.0 Introduction

Metabolic homeostasis reflects the complex output of endocrine, autonomic, and behavioral control circuits that extend throughout the central nervous system (CNS). Brain regions that control energy intake and expenditure are privy to continuous interoceptive feedback from the body that can modulate appetite, satiety, digestion, and metabolism. Interoceptive signals

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from the gastrointestinal tract and associated digestive viscera are delivered to the brain primarily by vagal afferents that terminate centrally within the medullary dorsal vagal complex (DVC), comprising the dorsal motor nucleus of the vagus (DMV), nucleus of the solitary tract (NST), and area postrema (AP) (Rinaman, 2003a). In addition to vagal inputs from gastrointestinal and other thoracic and abdominal viscera, DVC neurons receive direct and indirect interoceptive signals from olfactory, glossopharyngeal, trigeminal, facial, and spinal afferent systems. A strong topography is evident in the terminal arborizations of primary visceral afferents, with inputs from the gut terminating within the caudal medial NST (Altschuler et al., 1989; Shapiro and Miselis, 1985). In addition to synaptic inputs, the AP and a significant portion of the caudal medial NST contain fenestrated capillaries, allowing blood-borne factors (e.g., toxins, cytokines, hormones, and osmolytes) to alter local neural activity within the DVC and other brainstem targets (Cunningham et al., 1994).

A major product of integrated DVC neural activity is the modulation of autonomic vagal parasympathetic outflow to the stomach, small intestine, pancreas, and other digestive viscera (Altschuler et al., 1992). In addition, and as summarized in this review, ascending axonal projections from neurons within the caudal medial (i.e., gastrointestinal) NST target virtually every pontine, diencephalic, and telencephalic circuit node that has been implicated in the central control of energy homeostasis (Horst and Streefland, 1994), highlighting gastrointestinal interoception as a potentially critical modulator of neural circuit activity throughout the brain. Ample evidence supports the view that descending projections from hypothalamus to caudal brainstem provide critical control over the initiation and termination of food intake and feeding-related autonomic adjustments (Berthoud, 2002; Berthoud et al., 2006; Coll et al., 2007; Smith, 2000; Smith, 2003; Woods and D'Alessio, 2008; Zheng et al., 2005). Ascending projections from NST to hypothalamus are clearly involved in regulating hormone release from the anterior and posterior pituitary in response to gastrointestinal and other visceral sensory signals (Rinaman, 2007). Conversely, the influence of these ascending projections in regulating food intake is less firmly established (Luckman and Lawrence, 2003; Renner et al., 2010). Appetite and satiety are clearly modulated both by external (i.e., environmental) and internal (i.e., physiological) contexts, and, therefore, are only loosely dependent on past or current visceral sensory feedback signals.

Results from neuroanatomical studies performed primarily in rats have revealed potential pathways by which visceral sensory feedback signals can reach the hypothalamus and limbic forebrain, and thereby potentially affect the ways in which these forebrain regions control food intake. This article begins with an overview of ascending axonal projections from neurons within the caudal visceral NST to higher brain regions in adult rats. For this purpose, projections were identified using a standard anterograde tracer microinjected into the caudal NST, and also by immunocytochemical localization of glucagon-like peptide-1 (GLP-1). GLP-1-positive fibers within the brain arise exclusively from non-noradrenergic (NA) neurons within the caudal visceral NST and closely adjacent reticular formation (Larsen et al., 1997; Merchenthaler et al., 1999; Rinaman, 1999b; Vrang et al., 2007), thereby providing a clear view of ascending pathways arising from this small group of phenotypically distinct neurons. Projection pathways identified by these two approaches also are compared to the distribution of CNS neurons that become infected/labeled after inoculating the ventral stomach wall with H129, a neurotropic α-herpesvirus virus that transneuronally infects synaptically-linked chains of neurons in the anterograde direction (Rinaman and Schwartz, 2004).

The focus of this report is the anatomical organization and neurochemical phenotypes of ascending projections from the caudal gastrointestinal region of the NST in rats. Although some discussion of the hypothesized roles of these ascending projections is included where relevant, the reader is referred to several recent comprehensive reviews for more detailed

information regarding the involvement of particular diencephalic and limbic forebrain regions in the central neural control of food intake and energy expenditure (Berthoud, 2002; Berthoud, 2008; Broberger, 2005; Woods and D'Alessio, 2008).

2.0 Ascending visceral pathways: standard anterograde tracing from the noradrenergic (NA) region of the caudal NST

Since most neural pathways conveying interoceptive signals from body to brain involve a synaptic relay within the NST, a description of the central projections of NST neurons effectively reveals most CNS recipients of viscerosensory information (Bailey et al., 2006; Horst et al., 1989; Horst and Streefland, 1994; Ricardo and Koh, 1978), albeit without identifying the central targets of organ-specific sensory signals. A multitude of anterograde and retrograde tract-tracing studies, performed largely in rats, have demonstrated that neurons within the caudal visceral NST[1] have axons that project directly to a large number of central targets distributed across the medulla, pons, midbrain, hypothalamus, and limbic forebrain. Similarly, the present report documents the distribution of labeled axonal projections in a representative adult male Sprague-Dawley rat killed 10 days after unilateral iontophoretic injection of an anterograde neural tracer, Phaseolus vulgaris leucoagglutinin (PhAL, 2.5%) (Gerfen and Sawchenko, 1984), into the caudal visceral NST. Coronal brain sections (35 μm thick) were cut from the caudal medulla through the rostral extent of the corpus callosum, and a one-in-six series was processed for immunoperoxidase localization of PhAL. The distribution of PhAL-positive fibers was then mapped along the rostrocaudal neural axis using a light microscope equipped with a digital video camera and computerized tracing software.

Figure 1 depicts the caudal NST-centered PhAL injection site, and PhAL-positive fibers emanating from it. Immunoperoxidase labeling was so dense within the injection site (Fig. 1, gray shaded area) that it could not be accurately traced. To more precisely localize individual neurons that took up tracer within the injection site, an alternate set of sections from the same rat was processed for dual immunofluorescent localization of PhAL and the NA synthetic enzyme, dopamine beta hydroxylase (DbH) (Fig. 2). Neurons concentrating PhAL were restricted to the medial subnucleus of the NST at the rostrocaudal level of the area postrema (AP). The injection site overlapped the NST region that contains the A2 NA cell group, and a subset of PhAL-concentrating neurons were identified as DbH-positive (Fig. 2). Dual immunofluorescence labeling confirmed that the brainstem and forebrain distribution of PhAL-positive fibers overlapped with DbH immunolabeling; a few examples are shown in Figure 2. Conversely, the injection site in this rat did not label neurons within the medial commissural NST region (adjacent to the AP) that contains aldosterone-sensitive hydroxysteroid dehydrogenase-2 (HSD2) neurons. HSD2-positive NST neurons are implicated in the central control of sodium appetite (Geerling et al., 2006a;Geerling and Loewy, 2007), and appear to project to a discrete subset of the brain regions that receive input from NA and GLP-1-positive NST neurons (Geerling and Loewy, 2006).

The large majority of NST neurons that project to the hypothalamus and limbic forebrain are NA neurons of the overlapping A2/C2 cell groups (Sawchenko and Swanson, 1981; Sawchenko and Swanson, 1982a; Sawchenko and Swanson, 1982b), with remaining projection neurons primarily comprising smaller and separate populations of HSD2- and GLP-1-positive neurons (Geerling et al., 2006b; Larsen et al., 1997). NA projections from the caudal NST to higher brain regions are probably mostly glutamatergic, based on extensive colocalization of tyrosine hydroxylase (the rate-limiting enzyme for catecholamine

[1]The rostral gustatory NST gives rise to a largely distinct and more limited set of efferent projections (Norgren et al., 2003).

synthesis) and DNPI, the rat homolog of VGLUT2 (Stornetta et al., 2002). Despite a long scientific history supporting the involvement of central NA signaling in the central control of food intake and energy expenditure (Leibowitz et al., 1988; Ritter et al., 1975), it still is not clear whether or how NA inputs to the hypothalamus are involved in day-to-day regulation of energy balance. Conversely, there is ample evidence that NA inputs are invoved in hormonal and behavioral arousal responses to visceral stimuli. Medullary NA inputs to the hypothalamus provide critical control over the activity of stress-responsive corticotropin releasing hormone (CRH)-containing neurons within the PVH, at the apex of the HPA axis (Al-Damluji, 1988; Alonso et al., 1986; Banihashemi and Rinaman, 2006; Bienkowski and Rinaman, 2008; Gaillet et al., 1991; Kiss and Aguilera, 1992; Liposits et al., 1986; Rinaman, 2007). NA terminals also synapse directly onto thyrotropin releasing-hormone-positive neurons within the PVH (Füzesi et al., 2009), implicating NA pathways from the NST in metabolic responses to visceral stimuli. The results of phenotypically-specific lesioning experiments have demonstrated that NA inputs to the PVH are critical for the ability of systemic cholecystokinin-8 (CCK), lipopolysaccharide, lithium chloride, or yohimbine to activate Fos expression in PVH neurons, including CRH-positive neurons (Banihashemi and Rinaman, 2006; Bienkowski and Rinaman, 2008; Rinaman, 2003b; Rinaman and Dzmura, 2007). Interestingly, however, NA inputs to the PVH are unnecessary for the ability of CCK to inhibit food intake (Ritter et al., 2001). Indeed, the entire forebrain appears to be unnecessary for CCK-induced hypophagia (Grill and Smith, 1988). Although glucoprivic feeding induced by systemic 2-deoxyglucose is abolished in rats after bilateral destruction of NA inputs to the PVH (Ritter et al., 2001), it is unclear whether the same ascending pathways are important for the control of food intake under non-stressful, physiological conditions. Instead, it seems that ascending NA projections from the caudal NST may be recruited primarily during situations of real or perceived homeostatic challenge. As a case in point, experimental evidence supports the view that central prolactin releasing peptide (PrRP) signaling is involved in stress-related hypophagia (Lawrence et al., 2000; Lawrence et al., 2002; Lawrence et al., 2004), and PrRP is co-expressed by a subset of NA neurons within the NST that project to hypothalamic and limbic forebrain targets, including the PVH, paraventricular thalamic nucleus, DMH, medial preoptic area, peri-VMH, and BST (Renner et al., 2010; Yano et al., 2001).

The distribution of PhAL-positive fibers reveals that neurons within the caudal visceral NST project both contralaterally and ipsilaterally, although ipsilateral projections are more prominent (Figs. 3–10). Table 2 lists most of the brain regions that contained PhAL-positive fibers in this experimental case. The reader also is referred to similar anterograde tracing results reported earlier in adult rats (Horst et al., 1989;Horst and Streefland, 1994). Within the medulla, axons arising from neurons within the caudal visceral NST densely occupy the rostral gustatory NST (Fig. 3). Labeled axons also pass through the dorsal- and ventrolateral reticular formation (Figs. 3–4) while generally avoiding more medial regions of the medulla and pons. Within the pons, PhAL-positive fibers occupy the locus coeruleus (LC) and subjacent Barrington's nucleus (B; Fig. 4). Caudal visceral NST inputs to the medial and lateral parabrachial nuclei (PBN), including the Kölliker-Fuse (KF) subnucleus, are especially dense (Fig. 2B,Fig. 4–Fig. 5) (for more detail, see (Karimnamazi et al., 2002)). The PBN has at least 12 distinct subnuclei, some of which project to central targets that do not receive direct input from the NST (Fulwiler and Saper, 1984;Herbert et al., 1990;Moga et al., 1990;Saper and Loewy, 1980). For example, NST inputs to the internal lateral PBN, which provides a diffuse input to the intralaminar thalamic nuclei, may be involved in arousal responses to gastrointestinal and other visceral stimuli, while NST inputs to the external medial PBN may contribute to conscious appreciation of visceral sensation via thalamic relays to visceral cortex.

Within the midbrain, PhAL-positive fibers from the caudal visceral NST occupy the periaqueductal gray, particularly its ventral portion (Fig. 5). Labeled fibers also cluster within the serotonin-rich dorsal raphé (Fig. 5), and overlap the dopamine-rich ventral tegmental area (Fig. 6). The density of PhAL-positive fibers increases within the diencephalon (Figs. 7–8). Midline thalamic targets most notably include the paraventricular nucleus of thalamus. Hypothalamic targets include the lateral hypothalamic area (LHA), posterior hypothalamus, posterior periventricular nucleus, tuberomammillary nuclei (both dorsal and ventral), tuberal nucleus, dorsomedial nucleus, arcuate nucleus (ARH; Fig. 2C), paraventricular nucleus of the hypothalamus (PVH; including both magnocellular and parvocellular subregions; Fig. 2D), and supraoptic nucleus (SO). Interestingly, PhAL-labeled fibers tend to avoid the ventromedial hypothalamic nucleus (VMH), which has classically demonstrated roles in the central control of feeding and metabolism (King, 2006;Plata-Salaman, 1998). However, similar to neurons within the PVH and LHA (Jeanningros, 1984;Jin et al., 1993;Ueta et al., 1991), VMH neurons are activated by gastric distension via a vagal sensory pathway (Sun et al., 2006). NST inputs to VMH neurons may arrive on their distal dendrites which surround the VMH nucleus, where PhAL- (and GLP-1-)-positive fibers are present (see Fig. 12). More laterally within the subcortical telencephalon, PhAL-positive fibers cluster within the central nucleus of the amygdala and substantia innominata. More rostrally and dorsally, a small number of labeled fibers are present within the stria terminalis. Labeled fibers and varicosities also are observed within the dorsolateral horizontal limb of the diagonal band of Broca. Fibers and varicosities terminate densely within both the dorsal and ventral bed nucleus of stria terminalis (BST; Fig. 2E), and less densely within the medial and median preoptic nuclei, the organum vasculosum of the lamina terminalis, the medial septum, and the nucleus accumbens (ACB) (Figs. 9–10). The direct inputs from NST to ACB (ventral striatum) are of particular interest, given the prominent role of this limbic brain region in appetitive motivation (Kelley, 2004;Zheng et al., 2007).

No PhAL-positive fibers were observed within medial or lateral visceral cortex in this experimental case or in other similar PhAL tracing experiments from our laboratory, supporting previous reports that visceral sensory signals are relayed to the visceral cortices via the thalamus and other brain regions, including the LC and LHA. Wilder Penfield's cortical stimulation studies in humans revealed subjective sensations of oropharyngeal, esophageal, and gastrointestinal sensation organized in a topographic sensory homunculus within Brodmann's area 13, running ventrally from the tongue sensory area into the operculum and insular cortex (Penfield and Faulk, 1955). Cechetto and Saper reported a similar topographic pattern of visceral sensory responses in rats, involving regions of insular cortex that corresponded to viscerotopically organized inputs from the thalamus (Cechetto and Saper, 1987). In addition, the LC, LHA, and midline thalamic nuclei each have direct but diffuse cortical projections that likely participate in arousal and overall cortical "tone," and each of these regions receives visceral sensory input relayed directly from the caudal visceral NST in rats (Figs. 4, 7–8). In addition to receiving direct inputs from the caudal medial NST, the basal forebrain cholinergic corticopetal system also receives inputs relayed via the nucleus paragigantocellularis and LC (Bernston et al., 1998; Berntson et al., 2003). This cholinergic system is implicated in cortical arousal, attention, and anxiety, and is considered a widespread regulatory modulator that serves to enhance or amplify cognitive processing.

3.0 Ascending projections from the caudal visceral NST: immunocytochemical localization of GLP-1

GLP-1 is expressed by a relatively small number of neurons located within the caudal visceral NST (Fig. 11) and adjacent dorsal reticular formation (Jin et al., 1988;Larsen et al.,

1997;Merchenthaler et al., 1999). GLP-1-positive neurons are not adrenergic, but co-express β inhibin 1, somatostatin, and met-enkephalin (Sawchenko et al., 1990). Their ascending axonal projections largely parallel NA projections from the caudal NST. Indeed, all brain regions that contain GLP-1-positive fibers and terminals in adult rats also contain DbH-immunopositive fibers and terminals; however, the converse is not always true (personal observation), as the caudal NST is not the only source of NA fibers and terminals within the brain.

Central GLP-1 signaling pathways are implicated in the central control of food intake, glucose homeostasis, and HPA axis and autonomic responses to stress (Holst, 2007; Imeryuz et al., 1997; Kinzig et al., 2003; Nakade et al., 2007; Rinaman, 1999a; Sandoval et al., 2008; Tang-Christensen et al., 2001; Turton et al., 1996). GLP-1-positive terminals robustly innervate corticotropin releasing hormone (CRH)-positive neurons within the hypothalamic PVH (Sarkar et al., 2003), the apex of the HPA stress axis, as well as oxytocin (but not vasopressin) -positive neurons within the PVH and supraoptic nucleus (Rinaman and Rothe, 2002). GLP-1 modulates the activity of hypocretin/orexin-positive (but not melanin-concentrating hormone-positive) neurons within the LHA (Acuna-Goycolea and Pol, 2004), implicating GLP-1 signaling in behavioral arousal and reward-based feeding (Borgland et al., 2009; Cason et al., 2010 (in press)). In addition to their prominence within the PVH and LHA, GLP-1 receptors also are located within the ARH, along with GLP-1-positive fibers (see Fig. 12) (Alvarez et al., 1996; Merchenthaler et al., 1999).

GLP-1-positive NST neurons that project to the hypothalamus are activated by interoceptive stimuli that stimulate the HPA stress axis and also inhibit food intake in rats (Rinaman, 1999b), and central antagonism of GLP-1 receptors is sufficient to attenuate the hypophagic effect of lithium chloride (Rinaman, 1999a; Seeley et al., 2000). It remains unclear whether GLP-1 signaling within the forebrain, brainstem, or both regions is important for the control of food intake under natural conditions. Food intake is inhibited in rats after lateral or third ventricular administration of synthetic GLP-1 or agonist, or after microinjection of GLP-1 or agonist into the PVH, LHA, DMH, or VMH (Donahey et al., 1998; Schick et al., 2003). While these observations do not constitute evidence that endogenous GLP-1 plays a role in day-to-day body energy homeostasis, the available evidence does support the view that GLP-1 signaling pathways participate in stress-related hypophagia and activation of the HPA axis.

As seen in Table 2, every brain region that contained labeled fibers after PhAL injection into the caudal visceral NST also contained GLP-1-positive fibers. The distribution of GLP-1-positive fibers within the diencephalon and telencephalon is illustrated in Figure 12. As previously reported, GLP-1 positive fibers are relatively dense within the PVH (Fig. 13C), ARH (Fig. 13B), SO, DMH (Fig. 13A), PVT, and dorsal and ventral BST (Larsen et al., 1997;Merchenthaler et al., 1999;Rinaman, 1999b;Sarkar et al., 2003) (Fig. 12). GLP-1-positive fibers also occupy the ventral striatum (ACB; Fig. 13D).

4.0 Ascending gastric sensory pathways: viral transneuronal anterograde tracing

Standard anterograde and retrograde tracing techniques are useful tools with which to survey the inputs and outputs of various brain regions. However, light microscopic tracing using PhAL or other standard tracers cannot by itself demonstrate synaptic connections between labeled projection neurons and their targets. Further, PhAL tracing experiments like the one illustrated in Figures 1–10 cannot discriminate projections that carry gastrointestinal-related signals from those that carry cardiovascular or other visceral sensory information. In an attempt to isolate the postsynaptic targets of gastric-specific ascending sensory projections,

we turned to a novel transneuronal anterograde tracer, the H129 strain of herpes simplex virus-1 (Dix et al., 1983). H129 undergoes anterograde transneuronal transport in cebus monkeys after inoculation of primary motor cortex (Kelly and Strick, 2003; Zemanick et al., 1991) and in mice after inoculation of tooth pulp (Barnett et al., 1995) or the vitreous body of the eye (Sun et al., 1996). We found that H129 also has utility as an anterograde transneuronal viral tracer in rats, effectively revealing CNS regions that receive relayed gastric viscerosensory input (Rinaman and Schwartz, 2004). After H129 inoculation of the ventral stomach wall in adult male Sprague-Dawley rats, H129-immunopositive cells and fibers were present within the medullary DVC (including the caudal medial NST and medial DMV), thoracic spinal dorsal root entry zone, and thoracic spinal laminas I and II. In rats with longer post-inoculation survival times, additional spinal, brainstem, diencephalic, and telencephalic regions contained H129-positive cells (summarized in Table 2). Interestingly, some of the brain regions that contained transneuronal H129 labeling do not appear to receive direct projections from the caudal visceral NST, as evidenced by the absence of both PhAL anterograde labeling and GLP-1 immunopositive fibers in those regions (Table 2). For example, H129 labeling within visceral cortices presumably arose from the thalamus, LHA, and/or other brain region that contained H129-positive neurons and projects to visceral cortex. Further, transneuronal H129 transport from the stomach wall included ascending spinal visceral pathways in addition to pathways arising from the DVC, and so some H129 labeling is likely of spinal origin.

It seems curious that relatively little transneuronal H129 infection was observed within the ventrolateral BST, a forebrain region that receives particularly dense NA (Banihashemi and Rinaman, 2006; Myers et al., 2005) and GLP-1 inputs from the caudal NST (Fig. 12). H129 labeling also was not obvious within the ACB, another direct target of NA and GLP-1-positive projections from the caudal NST. Speculatively, the absence of H129 labeling in these regions may be due to a relatively low density of synaptic contacts. Transneuronal H129 infection depends on the presence of synaptic contacts for viral transport to occur from axon terminal to postsynaptic target; therefore, H129 transneuronal tracing cannot reveal forebrain neurons that are subject to visceral sensory modulation through local “paracrine” release of neurotransmitter from axon varicosities. Indeed, fewer than 20% of NA axon terminals within the ventrolateral BST form synaptic contacts (Phelix et al., 1992), and a hallmark of NA terminals within the medial PVH, ventrolateral BST, and other forebrain regions that receive dense inputs from the A2 region of caudal NST is the predominance of axonal varicosities that do not form synaptic contacts (Balcita-Pedicino and Rinaman, 2007). An absence of synaptic contacts, however, does not indicate an absence of neural signal transmission.

5.0 Conclusion

The CNS is privy to a plethora of peripheral neural and humoral signals that reflect current digestive status and energy availability, energy needs, and energy stores. Body energy homeostasis depends on the organism’s ability to integrate and respond adaptively to these signals by modulating current and future energy intake and expenditure. Clearly, descending projections from the hypothalamus and limbic forebrain to the DVC and other caudal brainstem regions are critical in the central control of digestion and feeding. The robust anatomical representation of reciprocal projections from the caudal visceral NST to feeding-related regions of the hypothalamus and limbic forebrain supports the view that these projections also are important in the central control of food intake. However, the available evidence indicates that signals carried to the forebrain may be important primarily for arousal and coordination of physiological and behavioral (i.e., hypophagic) responses to homeostatic challenge, rather than for modulating feeding and/or energy expenditure on a day-to-day basis. Future studies should challenge this assessment to determine whether or

not it is accurate, and to further reveal the physiological importance of visceral sensory signaling from gut to brain.

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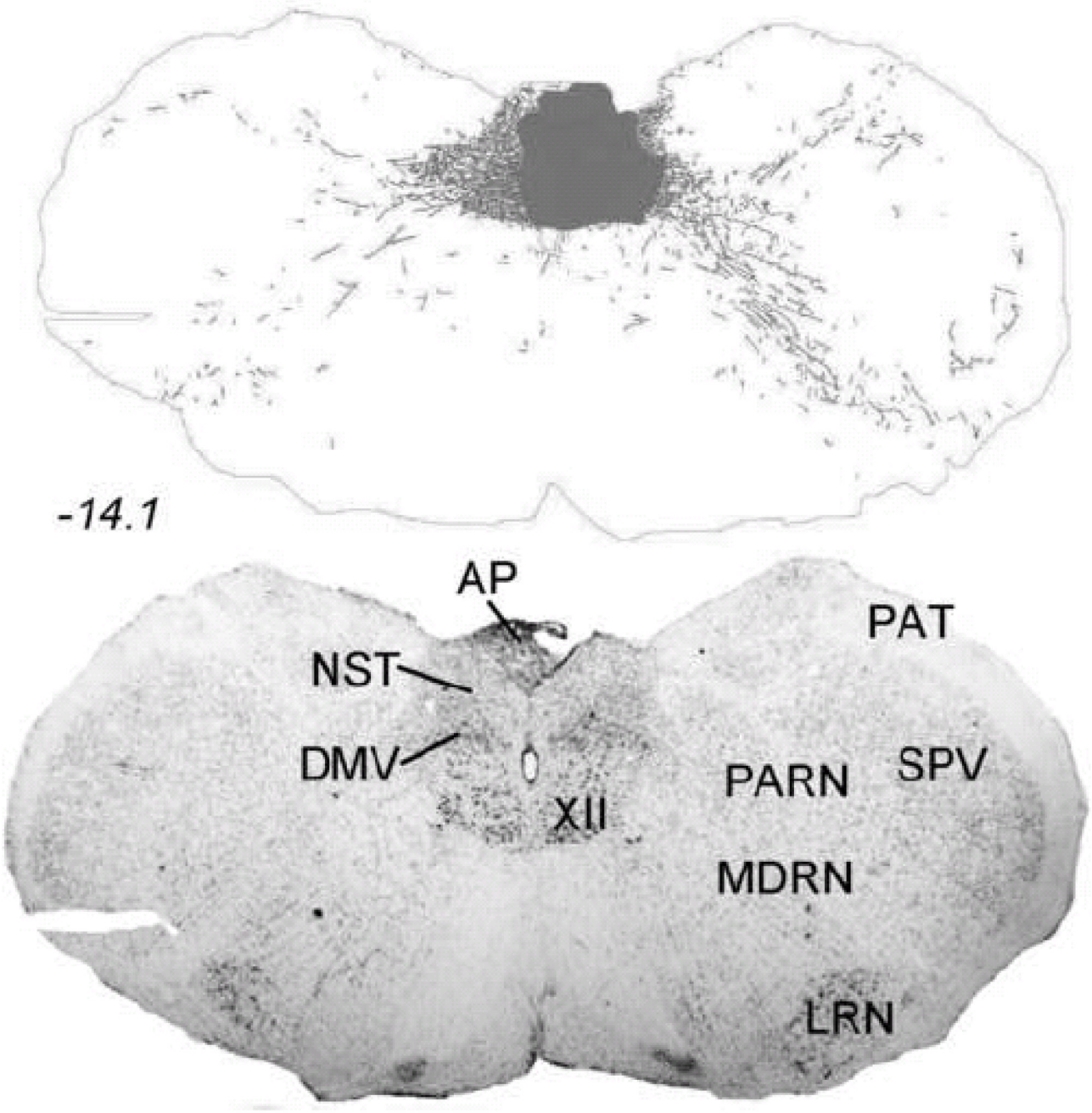

Figure 1.

Iontophoretic PhAL injection site within the caudal DVC in an adult male Sprague-Dawley rat. The dark gray shaded area in the upper panel depicts the region of the tracer injection site, which contained PhAL immunoperoxidase labeling that was too dense to accurately draw. See Figure 2 for immunofluorescence labeling of PhAL-concentrating neurons in an adjacent tissue section. Labeled fibers throughout the rest of the section in the upper panel (and in Figures 3–10) arise from PhAL-concentrating neurons located within the injection site. The lower panel is a nearby Nissl-stained tissue section from the same rat. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). See Table 1 for abbreviations.

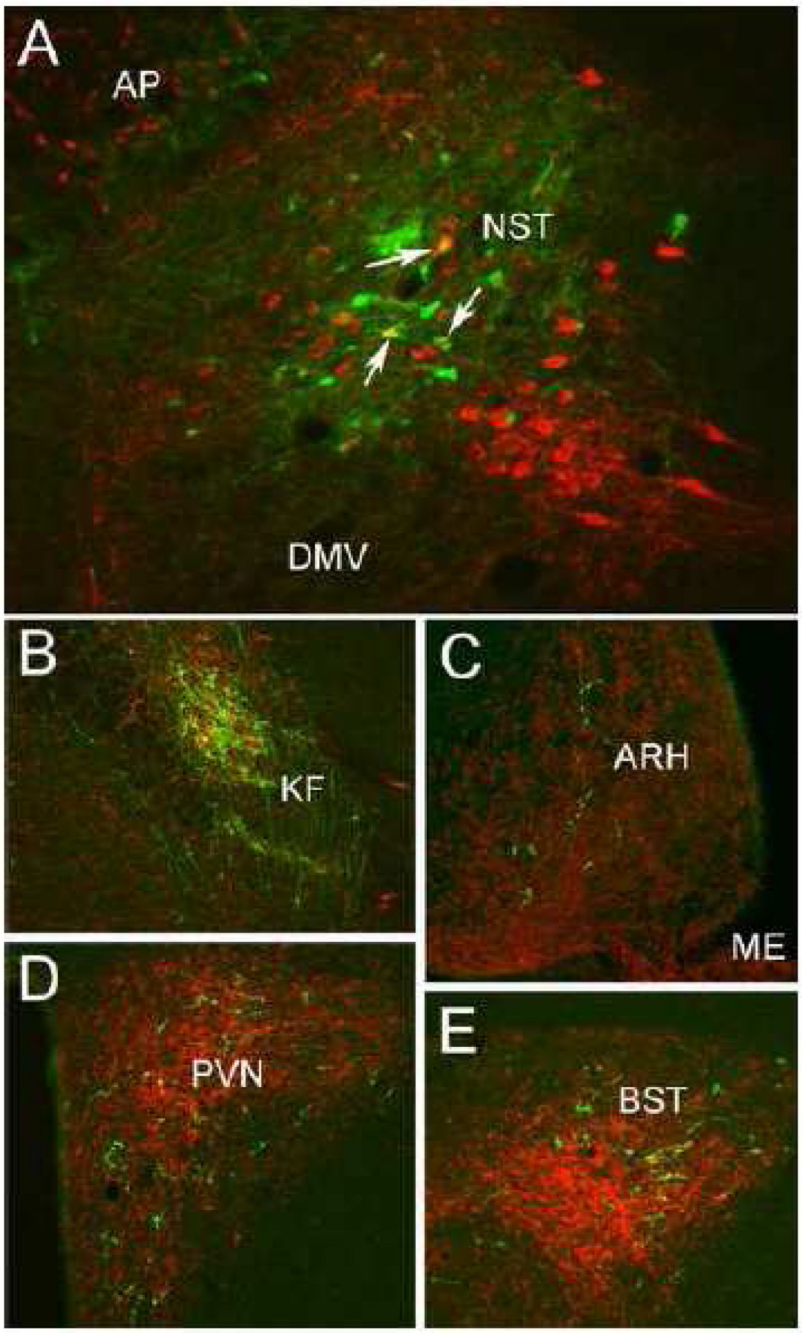

Figure 2.
Dual immunofluorescent localization of PhAL (green) and the noradrenergic synthetic enzyme, DbH (red). A: Individual NST neurons concentrating PhAL (green) within the iontophoretic tracer injection site (see Figure 1). A subset of these PhAL-positive neurons are DbH-positive (arrows point out 3 examples). B: PhAL-labeled fibers within the KF subregion of the lateral parabrachial nucleus. C: PhAL-labeled fibers within the hypothalamic ARH. D: PhAL-labeled fibers within the PVN. E: PhAL-labeled fibers within the BST. Note that each photomicrograph depicts PhAL and DbH immunofluorescent labeling photographed at only one focal plane through the section. See Table 1 for abbreviations.

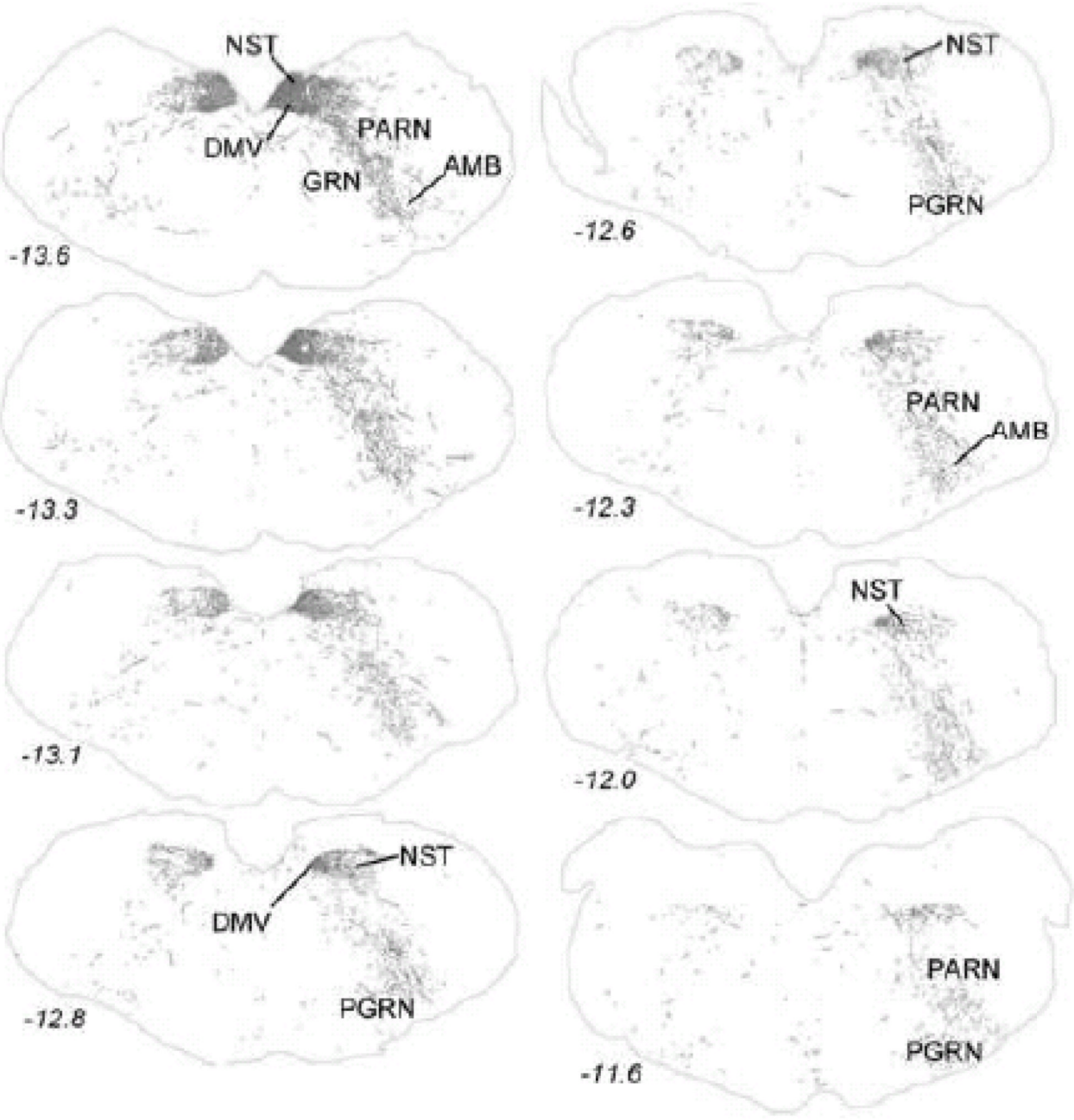

Figure 3.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to more rostral regions of the medulla. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). Upper left = caudal, lower right = rostral. See Table 1 for abbreviations.

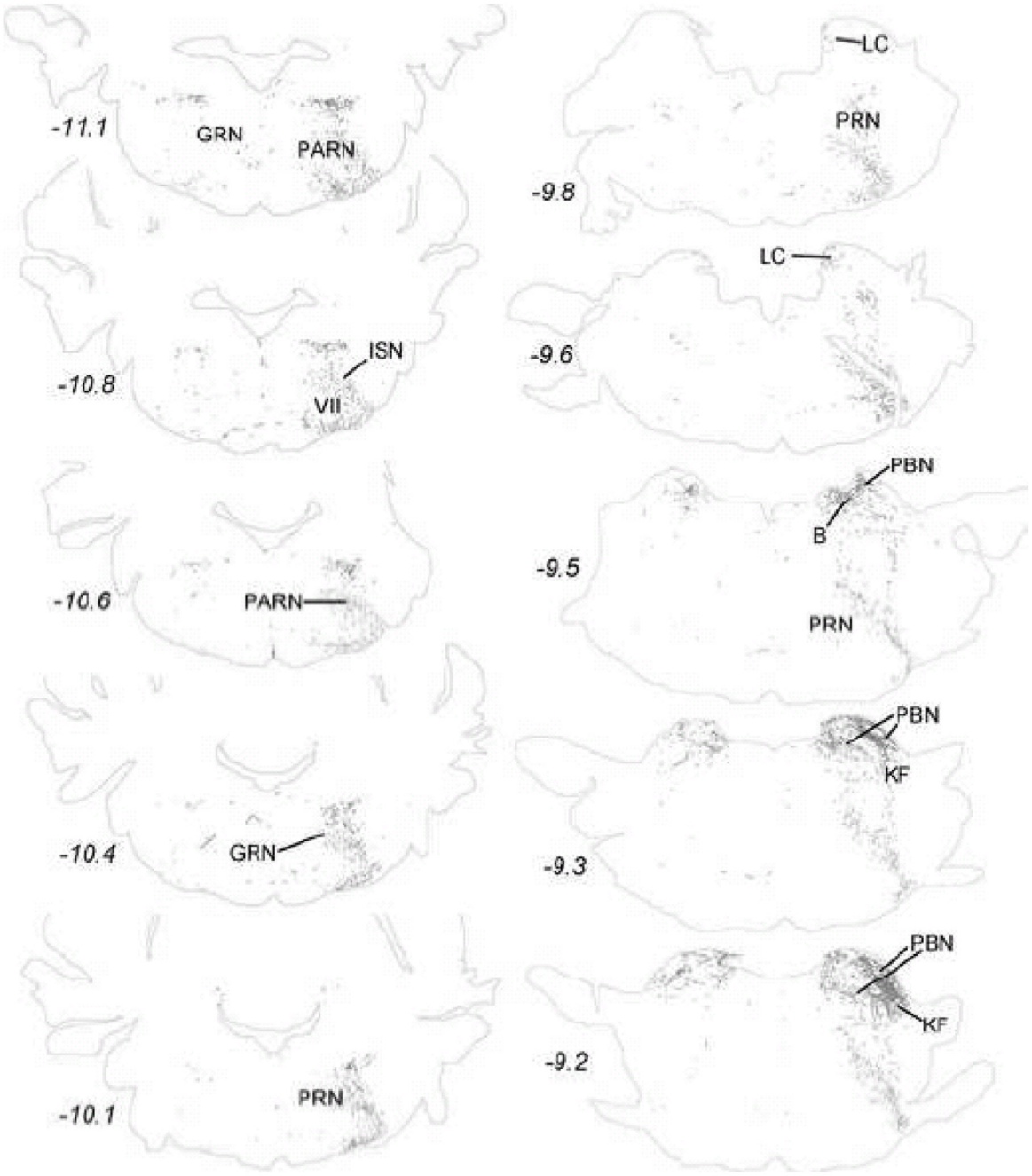

Figure 4.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to the pons. The approximate rostro-caudal locations of each section relative to bregma are indicated, based on a standard rat brain atlas (Swanson, 2004). Upper left = most caudal, lower right = most rostral. See Table 1 for abbreviations.

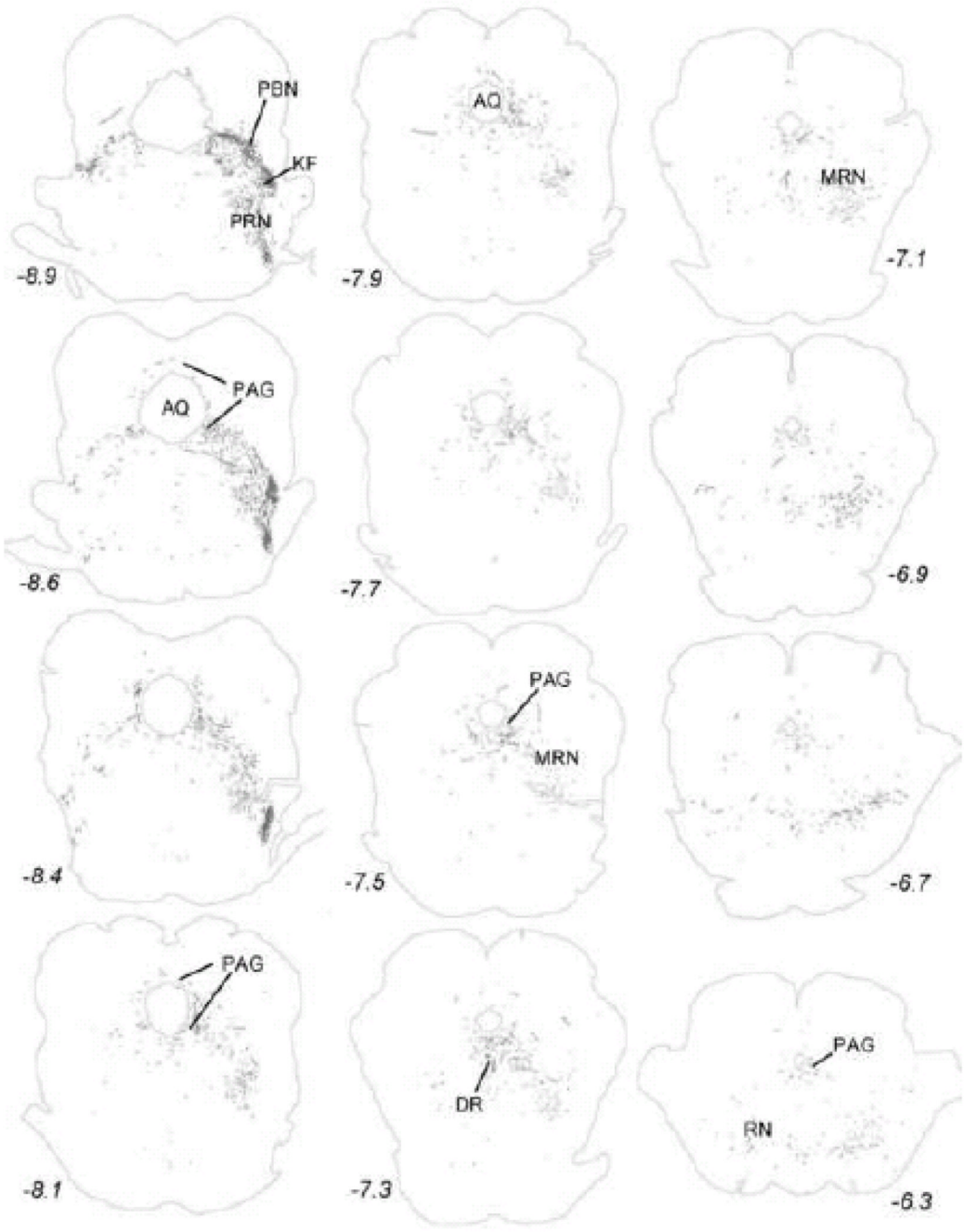

Figure 5.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to more rostral regions of the pons and midbrain. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). Upper left = caudal, lower right = rostral. See Table 1 for abbreviations.

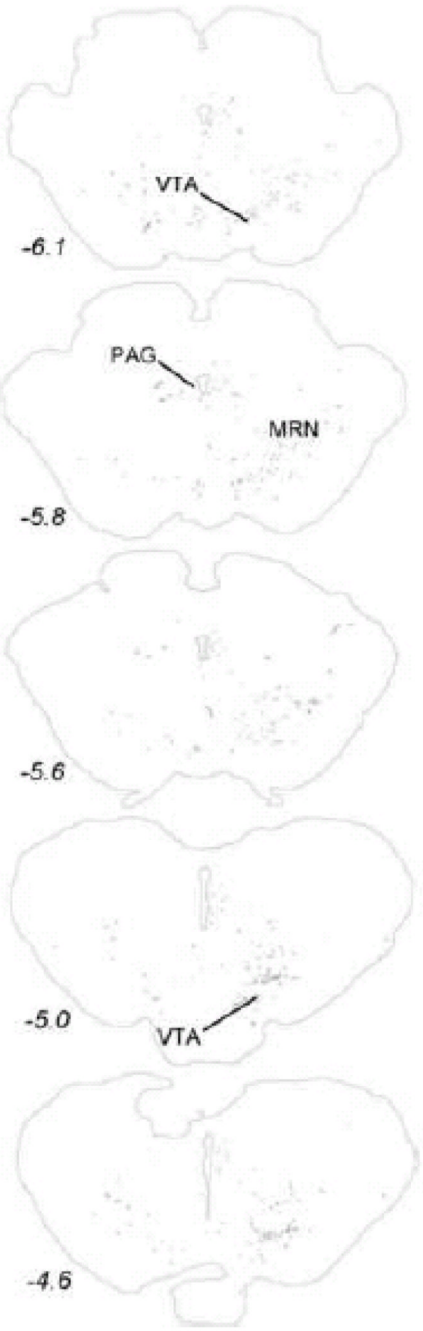

Figure 6.
Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to more rostral regions of the midbrain and caudal hypothalamus. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). Top = caudal, bottom = rostral. See Table 1 for abbreviations.

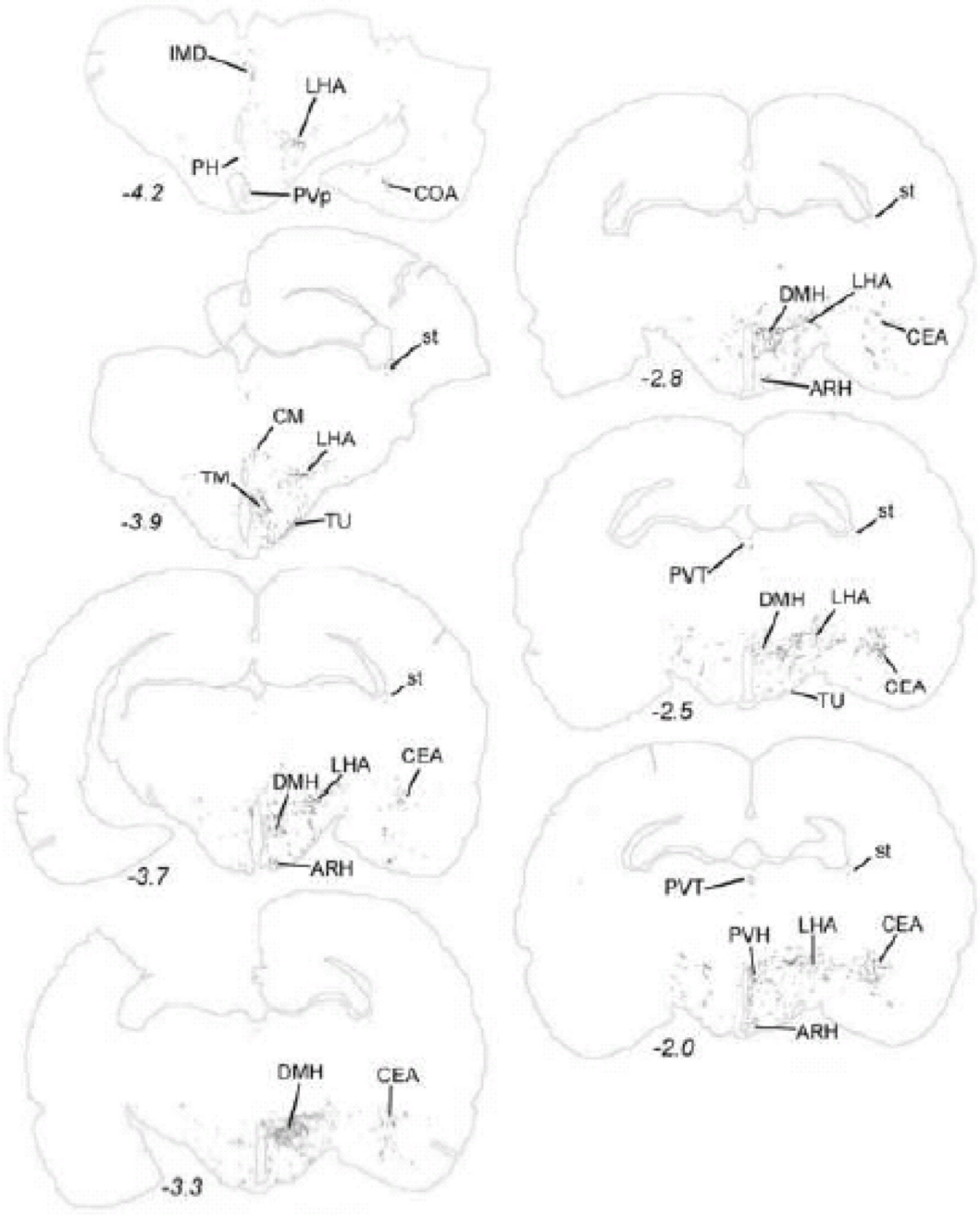

Figure 7.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to the hypothalamus and amygdala. The approximate rostro-caudal level of each section (relative to bregma) is indicated, based on a standard rat brain atlas (Swanson, 2004). Upper left = caudal, lower right = rostral. See Table 1 for abbreviations.

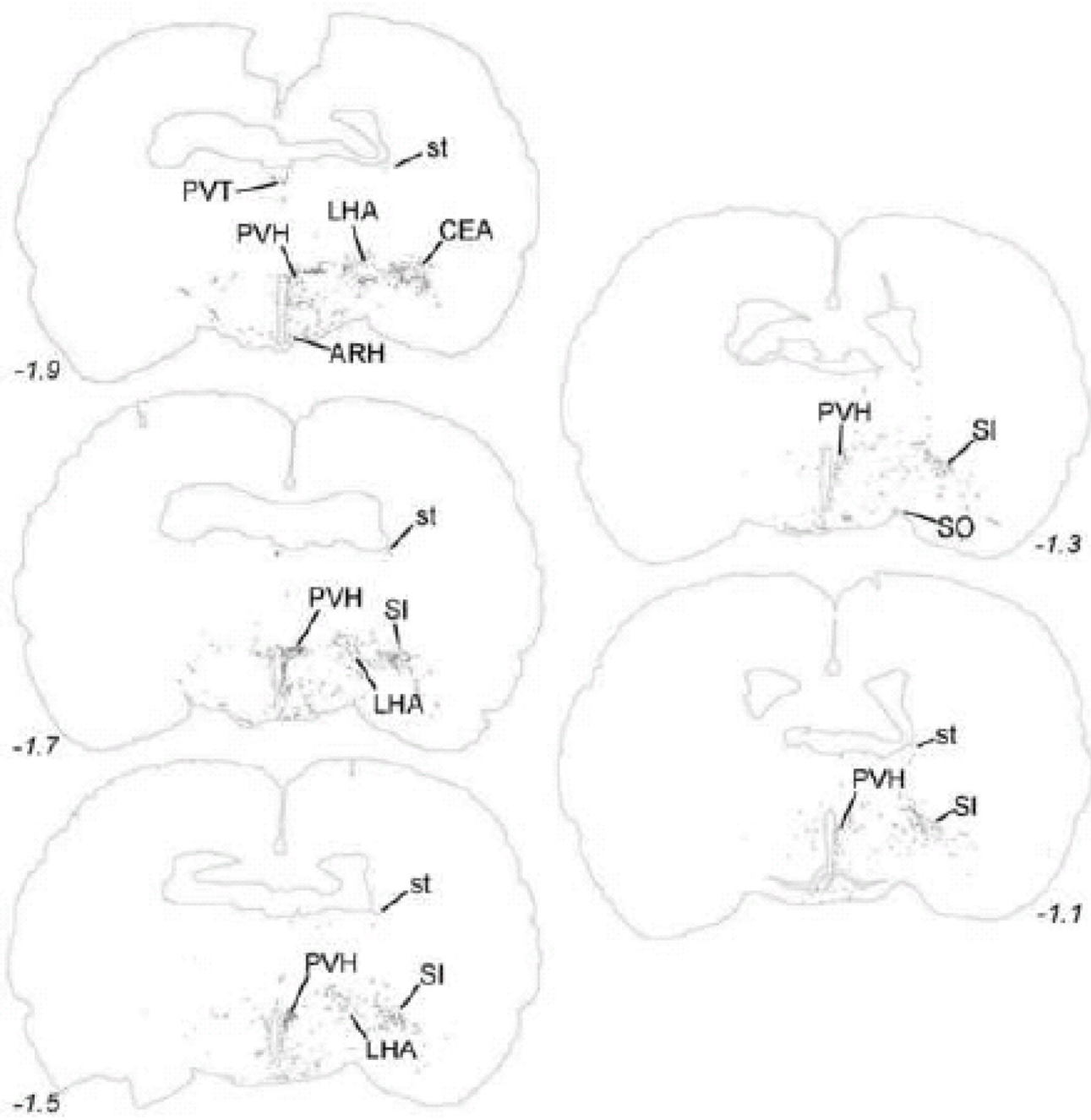

Figure 8.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to more rostral regions of the hypothalamus and amygdala. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). Upper left = caudal, lower right = rostral. See Table 1 for abbreviations.

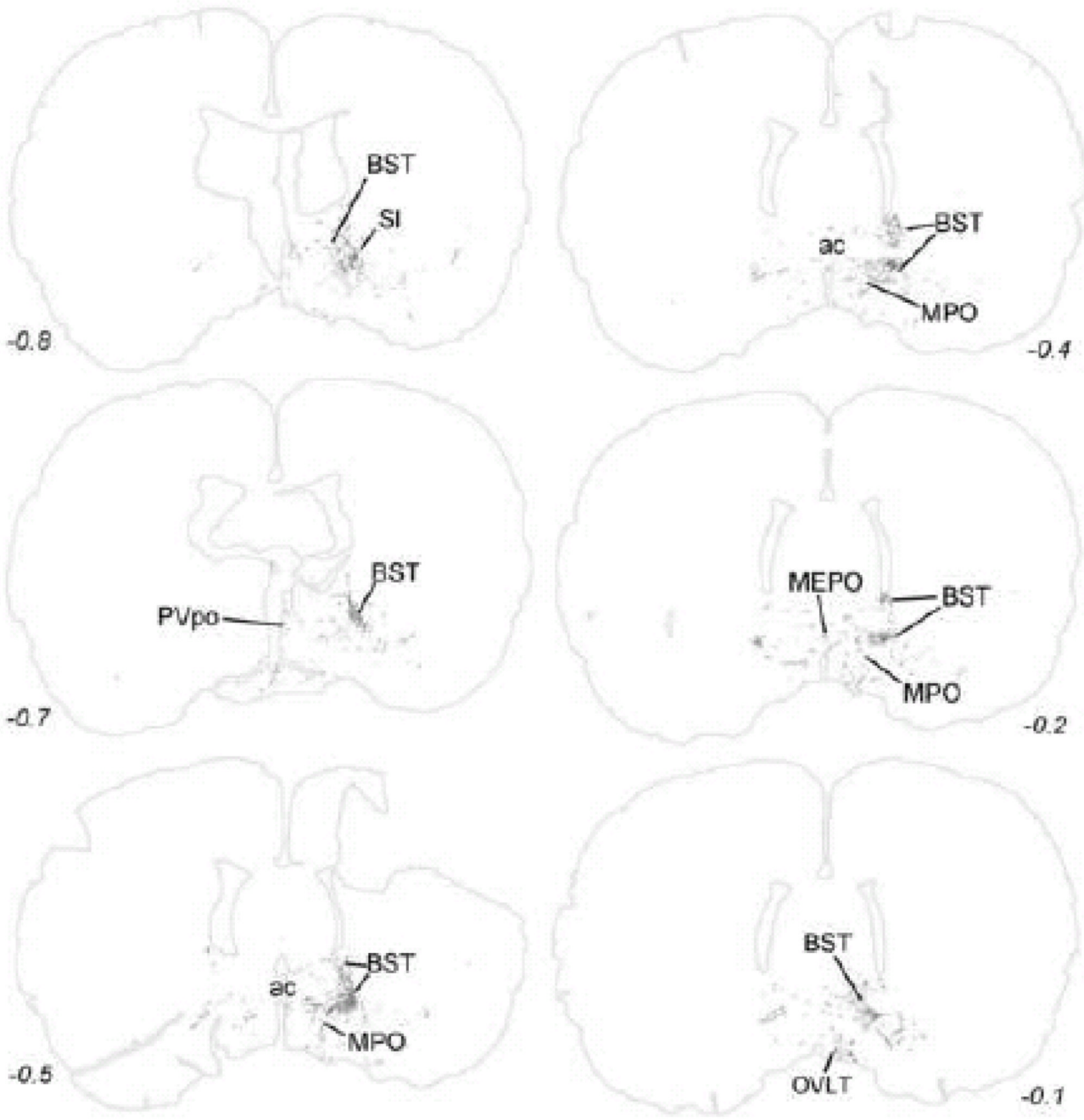

Figure 9.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to more rostral regions of the amygdala, substantia innominata, and bed nucleus of the stria terminalis. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated. Upper left = caudal, lower right = rostral. See Table 1 for abbreviations.

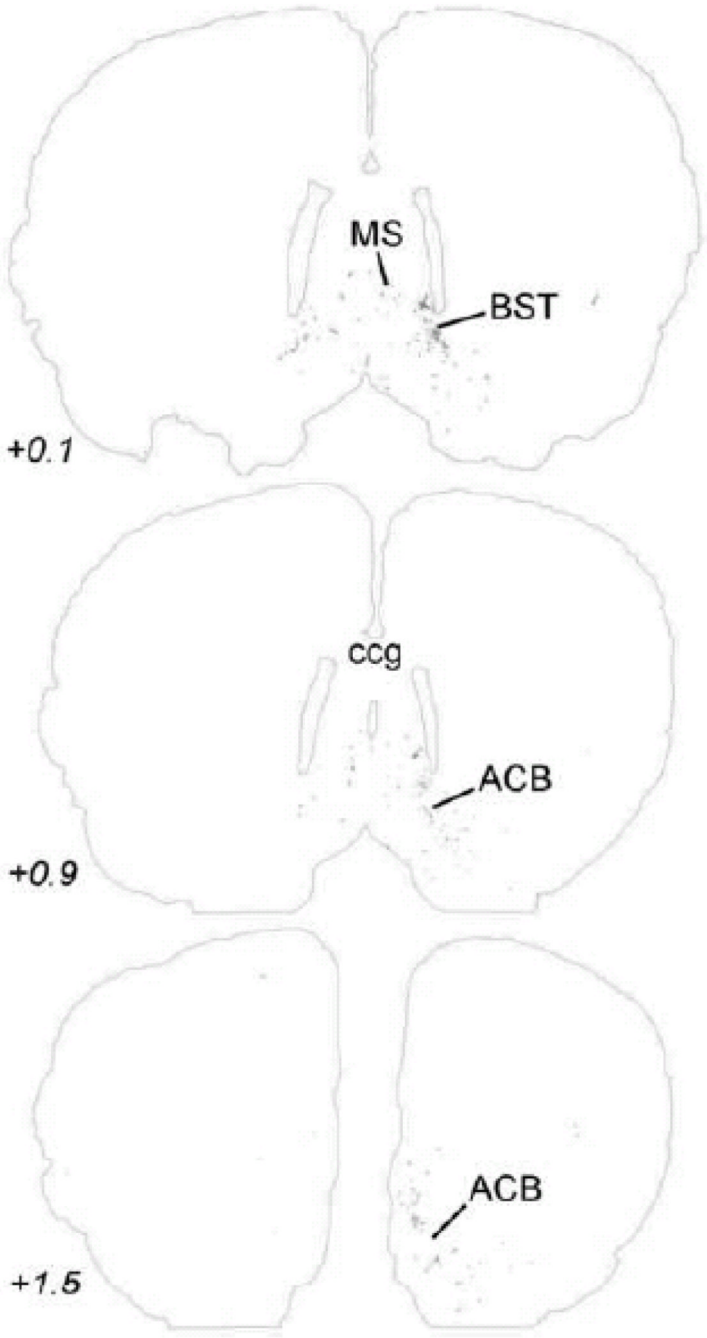

Figure 10.

Anterogradely transported PhAL from the caudal DVC (see Figures 1 and 2A for injection site) to more rostral regions of the bed nucleus of the stria terminalis and nucleus accumbens. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). Top = caudal, bottom = rostral. See Table 1 for abbreviations.

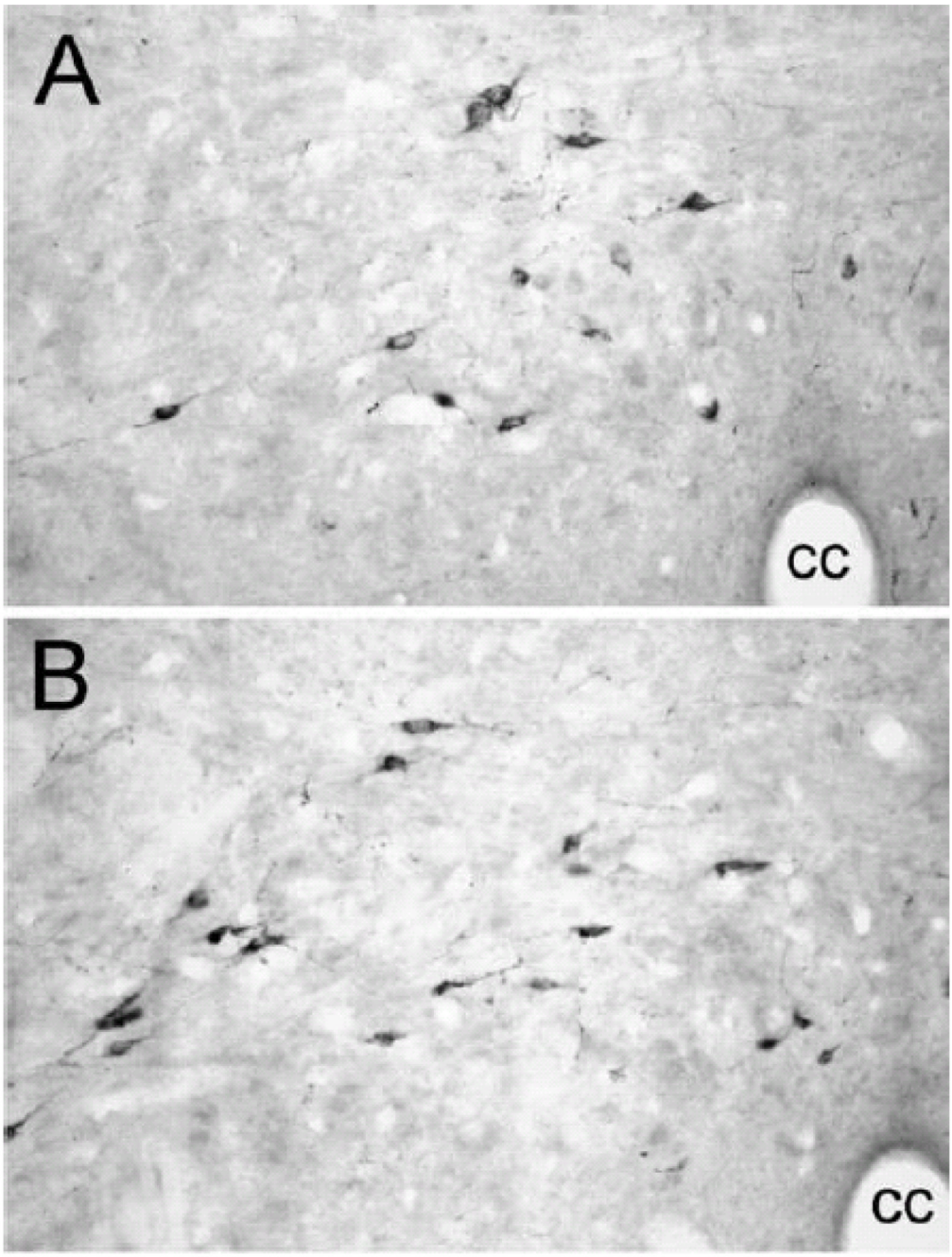

Figure 11.
GLP-1 immunoperoxidase labeling of neuron cell bodies within the caudal medullary DVC (A and B, approximately 15.0 and 14.0 mm caudal to bregma, respectively). See Table 1 for abbreviations.

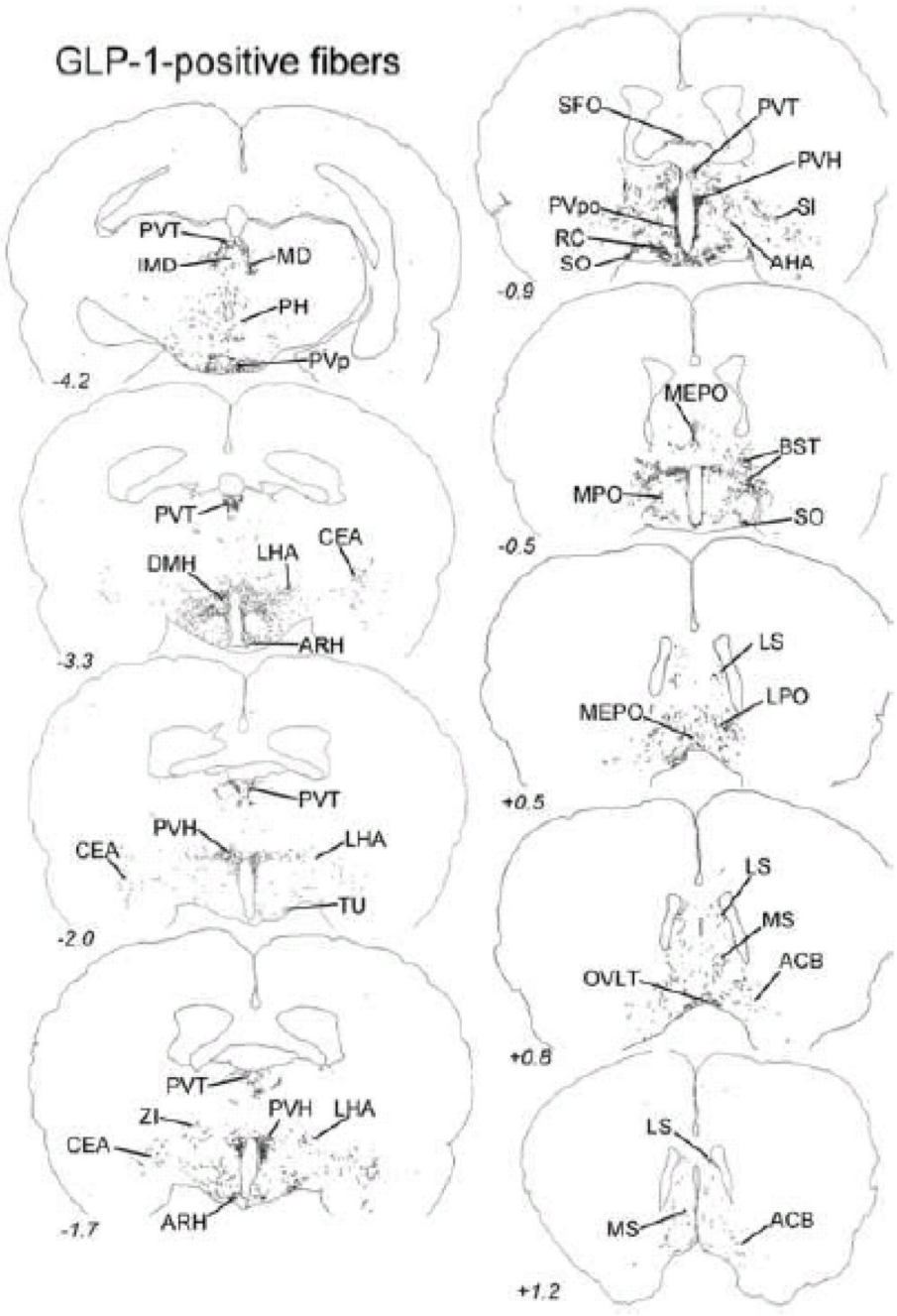

Figure 12.

Distribution of GLP-1-immunopositive fibers within the diencephalon and limbic forebrain. The approximate rostro-caudal level of each section (relative to bregma, in mm) is indicated, based on a standard rat brain atlas (Swanson, 2004). Top = caudal, bottom = rostral. See Table 1 for abbreviations.

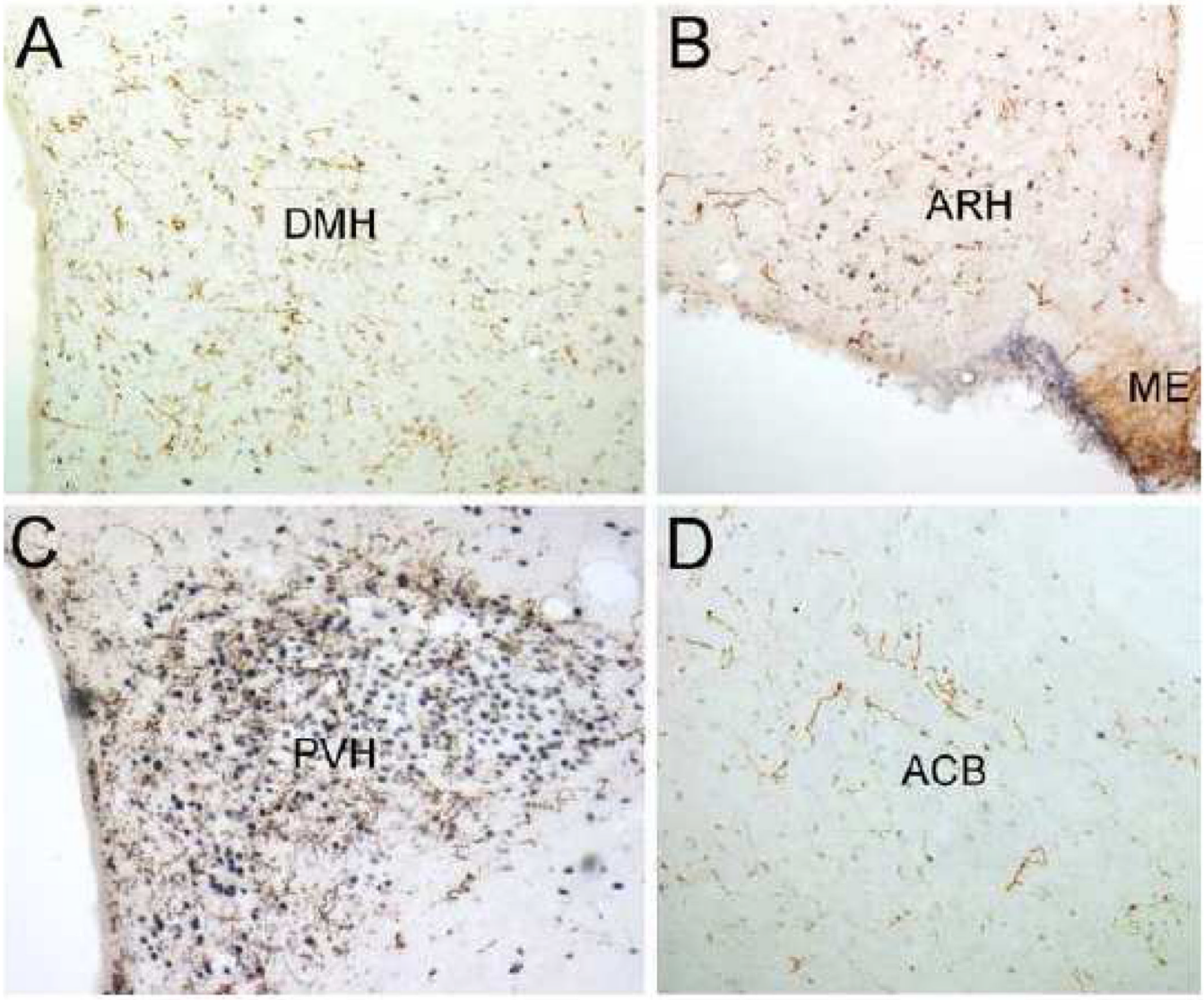

Figure 13.
GLP-1-positive fibers (brown) within the hypothalamic DMH (A), ARH (B), and PVH (C), and within the ventral striatum (D). Blue-black nuclear Fos immunolabeling is the result of lithium chloride (0.15M, 1% BW, i.p.) administration 90 min before perfusion fixation. See Table 1 for abbreviations.

Table 1

Abbreviations used in text and figures

Anatomical terminology after Swanson (Swanson, 2004).

3, third ventricle

ac, anterior commissure

ACB, nucleus accumbens

AHA, anterior hypothalamic area

AI, agranular insular cortex

AMB, nucleus ambiguus

AP, area postrema

ARH, arcuate nucleus of hypothalamus

B, Barrington's nucleus

BST, bed nucleus of stria terminalis

cc, central canal

ccg, corpus callosum genu

CEA, central nucleus of amygdala

CM, central medial nucleus of thalamus

CNS, central nervous system

COA, cortical amygdalar nucleus

DbH, dopamine β hydroxylase

DMH, dorsomedial nucleus of hypothalamus

DMV, dorsal motor nucleus of the vagus

DR, dorsal raphé nucleus

DVC, dorsal vagal complex (i.e., AP, NST and DMV)

GLP-1, glucagon-like peptide 1

GRN, gigantocellular reticular nucleus

HPA, hypothalamic-pituitary-adrenal

HSD2, 11-β-hydroxysteroid dehydrogenase type 2

HSV-1, herpes simplex virus type 1

IMD, intermediodorsal nucleus of thalamus

ISN, inferior salivatory nucleus

KF, Kölliker-Fuse subnucleus of PBN

LC, locus coeruleus

LHA, lateral hypothalamic area

LPO, lateral preoptic nucleus of hypothalamus

LS, lateral septum

MD, mediodorsal nucleus of thalamus

MDRN, medullary reticular nucleus

ME, median eminence

MEPO, medial preoptic nucleus of hypothalamus

MnPO, median preoptic nucleus of hypothalamus

MPO, medial preoptic area

MRN, midbrain reticular nucleus

MS, medial septum

NA, noradrenergic (i.e., DbH-positive)

NST, nucleus of the solitary tract

OVLT, organum vasculosum of the lamina terminalis

OT, oxytocin

PAG, periaqueductal gray

PARN, parvicellular reticular nucleus

PAT, paratrigeminal nucleus

PERI, perirhinal cortex

PGRN, paragigantocellular reticular nucleus

PH, posterior hypothalamus

PhAL, phaseolus vulgaris leucoagglutinin

PRN, pontine reticular nucleus

PrRP, prolactin-releasing peptide

PBN, parabrachial nucleus

PVH, paraventricular nucleus of hypothalamus

PVp, posterior periventricular nucleus of hypothalmus

PVpo, preoptic periventricular nucleus of hypothalamus

PVT, paraventricular nucleus of thalamus

RC, retrochiasmatic nucleus of hypothalamus

RN, red nucleus

SI, substantia innominata

SFO, subfornical organ

SO, supraoptic nucleus of hypothalamus

SPVC, spinal nucleus of trigeminal

st, stria terminalis

subLC, sucoeruleus

TM, tuberomammilary nucleus of hypothalamus

TU, tuberal nucleus of hypothalamus

VLM, ventrolateral medulla

VII, facial motor nucleus

VTA, ventral tegmental area

XII, hypoglossal motor nucleus

ZI, zona incerta

Table 2

Central targets of axonal projections from the caudal visceral NST identified in adult rats using three different approaches. See Table 1 for abbreviations. +, labeling present; ++, labeling moderate; +++, labeling dense.

CNS Division **NUCLEUS**	**PhAL-labeled axons from caudal medial NST**	**GLP-1-immunopositive fibers**	**H129 transported from stomach wall**
Medulla			
DMV	+++	+	+++
cNST	+++	+	+++
rNST	+++	+	+
PAT	+	+	++
RO	+	++	+
RPA	+	+	+
MDRN	++	+	+
PARN	++	+	++
SPV	+	+	+
AMB	++	++	++
PGRN	++	+	+
ISN	+	+	+
GRN	++	+	+
PRN	++	+	+
VII	+	+	+
Pons			
LC	+	+	+
subLC	+	++	++
B	++	++	+
PRN	++	+	+
PBN	+++	+++	++
KF	+++	+++	++
Midbrain			
PAG	++	+	+
MRN	+	+	+
DR	+	+	+
VTA	+	+	+
Diencephalon			
thalamus			
IMD	+	+	
CM	+	+	
MDm		++	+
PVT	++	+++	+

CNS Division **NUCLEUS**	**PhAL-labeled axons from caudal medial NST**	**GLP-1-immunopositive fibers**	**H129 transported from stomach wall**
hypothalamus			
PH	+	+	+
LHA	++	+	++
PVp	+	++	+
TU	+	+	+
TM	+	++	+
DMH	+++	+++	+
ARH	+	+	+
PVH	+++	+++	++
SO	+	++	
ZI	+	+	
RC		++	++
PVpo	+	++	+
MEPO	+	++	+
MPO	+	+	+
LPO		++	
SFO		++	
ME	+	++	
OVLT	+	++	
Telencephalon			
subcortical			
CEA	++	+	++
SI	++	++	+
dlBST	++	++	++
vlBST	+++	+++	+
MS	+	+	+
LS		+	+
ACB	+	+	
cortical			
PERI			+
AI			+