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Two physiologically distinct populations of neurons in the ventrolateral medulla innervate the locus coeruleus

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Recent anatomic studies indicate that the nucleus paragigantocellularis (PGi), located in the rostral ventrolateral medulla, strongly innervates the locus coeruleus (LC) while no such input derives from the more caudally located lateral reticular nucleus (LRN). In the present study, focal electrical stimulation of the LC was used to antidromically activate neurons in the ventrolateral medulla. A substantial number of PGi neurons were antidromically driven from the ipsilateral LC, while antidromic activation was virtually absent in LRN. Furthermore, several physiologic properties of antidromically driven cells in PGi define two populations within this group of neurons afferent to LC. These findings provide physiologic confirmation of an anatomically identified input to LC.

INTRODUCTION

The nucleus locus coeruleus (LC) in the rat consists of a small, dense collection of noradrenergic neurons in the dorsolateral pons that project extensively throughout the brain. Many facets of LC physiology and anatomy have been intensively studied, and various lines of evidence indicate that this nucleus may play an important role in attention and vigilance[4,5], as well as in the etiology of mental disorders, including psychoses and senile dementia of the Alzheimer's type[13,24]. Despite the high significance and numerous studies pertaining to this brain system, little is known about neural circuitry providing control of LC. Recent anatomic studies, however, have identified a major source of afferents to LC, originating in the ventrolateral medulla.

Initial studies employing retrograde transport of non-conjugated horseradish peroxidase (HRP) reported ventrolateral medullary projections to LC from the lateral reticular nucleus (LRN)[11,12]. Additional evidence for such a projection was obtained using anterograde transport of radiolabeled amino acids and retrograde transport of True blue[27,31]. However, our recent studies[6] employing retrograde transport of wheat germ agglutinin conjugated–HRP (WGA–HRP) revealed two prominent sets of afferents to LC: the nucleus prepositus hypoglossi located in the dorsomedial rostral medulla, and the nucleus paragigantocellularis (PGi)[1] located in the rostral ventrolateral medulla. Thus, these anatomic data indicate the ventrolateral medullary projections to LC originate in PGi, instead of the more caudally situated LRN. Our finding of a strong projection from PGi to LC, recently replicated by others[21], is compatible with additional findings that tritiated amino acids are transported into LC from the rostral, but not caudal, ventrolateral medulla[28].

In the present experiments, focal electrical stimulation of LC antidromically activated numerous neurons in PGi but not in the LRN, providing additional support for major innervation of LC from the more rostral medullary location, PGi. Interestingly, the physiologic properties of antidromically activated neurons indicate that at least two populations of PGi cells innervate LC. A preliminary report of some of

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this work has appeared elsewhere[16].

MATERIALS AND METHODS

Twenty-three male albino rats (300–400 g) were used in these experiments. Most animals were initially anesthetized with chloral hydrate (400 mg/kg, i.p.); anesthesia was maintained with injections of 30–40 mg/kg chloral hydrate administered as needed (approximately every 30 min) or by continuous administration using a motor-driven syringe pump. Two animals were anesthetized with 0.5–1.0% halothane in air via spontaneous respiration. Animals were mounted in a stereotaxic instrument with the incisor bar lowered to place the skull at a 15° angle relative to the horizontal plane. Body temperature was maintained at 36–37 °C with a feedback-controlled heating pad.

Stimulation electrodes, consisting of paired stainless steel microwires (50 μm-diameter), insulated except for bluntly cut tips, were implanted in LC (3.7 mm posterior to lambda, 1.2 mm lateral to midline, 5.7 to 6.5 mm ventral to skull surface) and cemented to adjacent skull screws. Electrophysiologic recordings from the wires during implantation were used to localize LC according to its characteristic spontaneous and sensory-evoked discharge patterns[10,17,35]. Two hypodermic needles (26 gauge) were placed in the contralateral medial hindpaw for s.c. electrical stimulation of the sciatic nerve.

For micropipette recordings, the incisor bar was lowered to place the skull at 24° relative to the horizontal plane. The skin and neck muscles overlying the medulla were reflected, and the occipital plate and dura covering the medulla and caudal cerebellum were removed. Extracellular recordings from individual neurons in LRN (0.5 mm posterior–1.5 mm rostral to obex, 1.5–2.1 mm lateral to midline) and PGi (1.8–2.7 mm rostral to obex, 1.5–2.4 mm lateral to midline) were obtained using glass micropipettes filled with a Pontamine sky blue solution (2–4 μm diameter, 10–20 MΩ impedance).

Bipolar electrical stimulation of LC (0.5 ms pulse duration, approximately 1 stimulus/s) was used to maximize detection of non-spontaneous afferent neurons during micropipette penetrations. Driven cells were considered antidromic if they met the following criteria: constant latency driving at threshold for activation, ability to follow twin pulse stimulation at frequencies of 200 Hz or greater, and (for spontaneously active neurons) collision of driven spikes with spontaneous impulses (see Fig. 2). In addition, cells were considered to be antidromically driven from LC only if their threshold for activation was below intensities that yielded jaw and facial twitching (which may reflect activation of trigeminal pathways adjacent to LC). Because facial twitching was typically associated with stimulus currents greater than 700 μA, but never below 500 μA, cells with antidromic thresholds in or above this range were not included in data analyses.

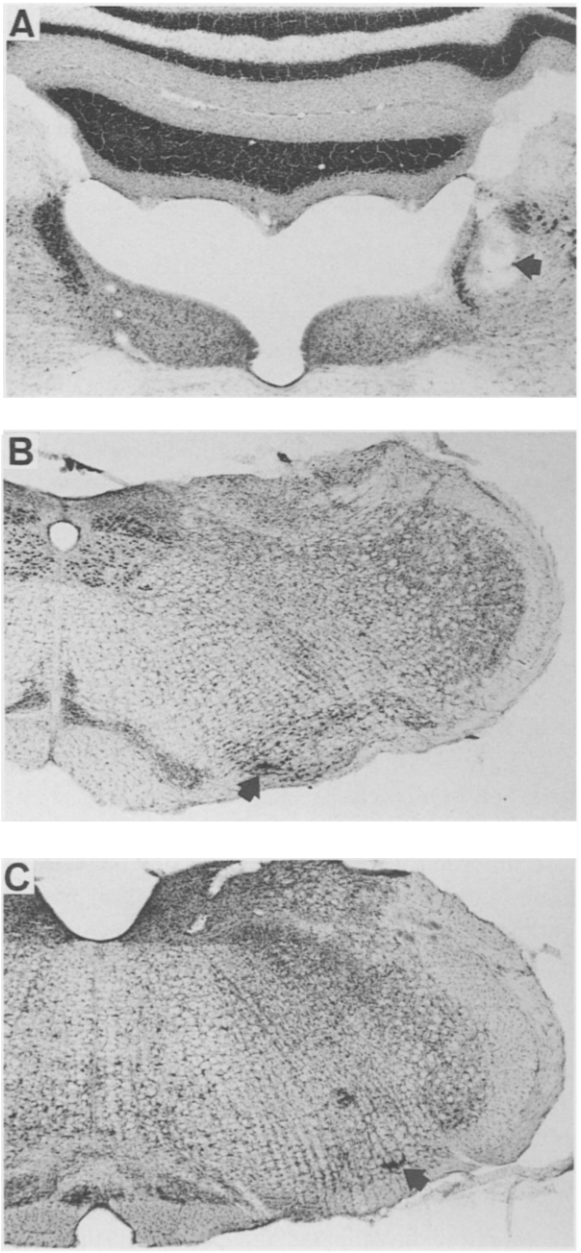
Dye was iontophoresed at the end of micropipette penetrations, or at the location of driven cells if encountered. Following experimental sessions, animals were deeply anesthetized, perfused with a formalin solution, and brains were removed for subsequent histologic analyses. All data reported here are from animals in which stimulation sites were histologically localized to LC and in which recording sites were confirmed for LRN and PGi (Fig. 1).

RESULTS

Recordings from LRN neurons. Forty-four neurons were encountered in 15 micropipette penetrations through LRN (7 animals; Fig. 1B). Most of these neurons spontaneously discharged in doublets and exhibited impulse waveforms that were entirely negative in unfiltered records. Only two of 40 cells tested were antidromically activated, at latencies of 1.0 and 1.8 ms. One of these cells, however, was located in the rostral pole of LRN, at the border of this nucleus and PGi. Three other cells were antidromically driven but with high thresholds for activation (in excess of 1.0 mA) and were therefore not considered to be driven from LC. Sixteen LRN cells were driven at variable latencies and did not exhibit collision between spontaneous and driven impulses. Such synaptic activation ranged from 1 to 8 ms. The threshold for synaptic activation of these cells was relatively high (mean = 707 μA), and was often accompanied by twitching of the jaw and facial muscles of the animal.

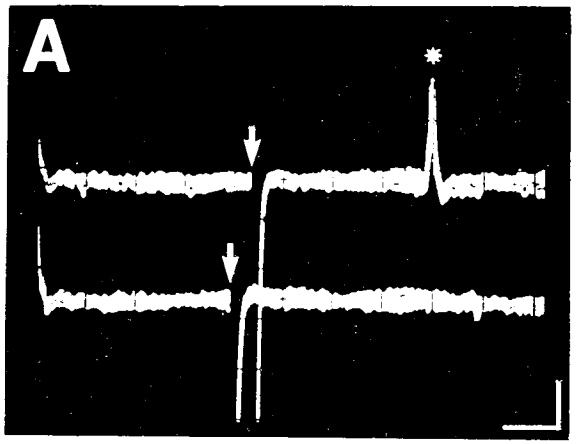
Recordings from PGi neurons. Ninety neurons were encountered in 44 penetrations through PGi (16 animals: Fig. 1C). Cells in this area were heteroge-

neous in terms of impulse waveforms, but the majority (86%) exhibited negative waveforms in unfiltered records with amplitudes less than 0.5 mV. Spontaneous discharge (observed in 64 cells) was also variable, ranging from 1 to 20 spikes/s. Thirteen cells exhibited rhythmic discharge related to respiration. These cells were encountered in the dorsal margins of PGi, similar to previous reports of respiratory neurons in PGi[9,19]. Twenty-six non-spontaneous cells were detected by synaptic or antidromic activation.



Of 79 PGi neurons tested, 20 were antidromically driven (Fig. 2) at a mean threshold of 339 μA. Antidromic latencies ranged from 1.5 to 21.0 ms with a mean ($\pm$ S.E.M.) latency of 8.6 $\pm$ 1.5 ms. A frequency distribution of antidromic latencies of spontaneous and non-spontaneous cells is depicted in Fig. 3. This plot reveals a bimodal distribution of antidromic latencies, such that the spontaneously active neurons yielded latencies distributed over longer values than those of the non-spontaneous cells. Six cells were spontaneously active and were driven at latencies ranging from 2.3 to 21 ms, with a mean latency of 14 $\pm$ 2.6 ms. Non-spontaneous cells (n = 14) were driven at significantly shorter latencies (range = 1.5–17.0 ms, mean = 6.1 $\pm$ 1.3 ms; $P < 0.02$, t-test). Furthermore, spontaneously active cells antidromically driven from LC typically exhibited impulse waveforms that were large and entirely positive (in excess of 1.0 mV in unfiltered records, 4 of 6 cells; Fig 2A), while those of non-spontaneous driven cells were negative and smaller in amplitude (less than 0.5 mV, each of 14 cells; Fig. 2B). The overall difference in antidromic latencies for these two groups categorized by waveform and spontaneous activity was more pronounced and reliable (17 $\pm$ 1.7 ms vs 6.1 $\pm$ 1.3 ms, respectively; $P < 0.002$, t-test) than for cells categorized by activity patterns alone (above). Thus, as shown in Fig. 3, differing spike waveforms superimpose with the bimodal distribution of antidromic latencies and spontaneous activity patterns. These results indicate that there are two physiologically distinct populations of PGi neurons afferent to LC: (1)

Fig. 1. Stimulation and recording sites. A: photomicrograph of a coronal section (Neutral red stain) taken through LC of an experimental rat brain. Lesion, at arrow, reveals placement of stimulation electrode tips in LC. For comparison, the intact LC contralateral is seen at the far left, just lateral to the IVth ventricle. Dorsal is at the top. B: coronal hemisection (midline at the left) taken through the caudal medulla posterior to the obex of an experimental rat brain. Dye spot (at arrow) was created at the end of a micropipette penetration through LRN. Large, darkly stained neurons of LRN are seen in the ventral aspect of the medulla, lying beteen the inferior olive and the spinal nucleus of the Vth nerve. C: similar section except through the rostral medulla (approximately 2 mm rostral to the obex) revealing location of dye spot (at arrow) marking recording site in PGi. All recording sites were histologically verified from such tissue sections. For calibrations, length of arrow in A = 160 μm, and arrows in B and C = 200 μm. For landmarks, refer to Fig. 4A.



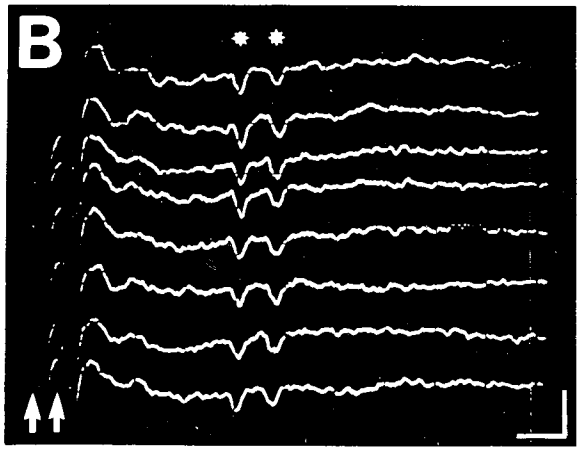


Fig. 2. A: collision test for a spontaneous PGi neuron driven from LC. Upper trace, LC stimuli (arrow) triggered 22 ms after spontaneous PGi impulses (trailing edge at far left) elicit constant latency spikes (21 ms, at star). Note large, positive impulse waveform typically exhibited by spontaneous PGi neurons antidromically driven from LC. Lower trace, driven spikes are occluded for similar stimuli triggered 19 ms after spontaneous impulses, indicating collision between spontaneous and driven spikes. Five superimposed sweeps of unfiltered records in each trace. Calibration: vertical bar = 0.5 mV, horizontal bar = 5.0 ms. B: high frequency activation of a non-spontaneous PGi neuron. Paired LC stimuli (at arrows, 1.5 ms interpulse interval) elicit constant latency spikes (stars). Note small, negative spike waveforms, characteristic of non-spontaneous PGi neurons driven from LC. Calibration: vertical bar = 0.5 mV, horizontal bar = 2.0 ms.

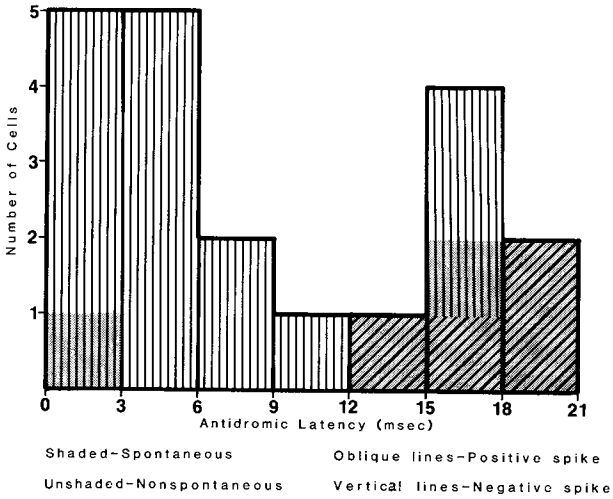


Fig. 3. Frequency histogram of antidromic latencies for PGi neurons. Note the wide range of latencies. Spontaneously active cells with positive waveforms exhibit longer latencies than non-spontaneous cells with small negative spikes (see text for details).

non-spontaneous, relatively fast conducting cells with small negative spikes and (2) slower conducting, spontaneously active cells with larger positive spikes (preferentially located in medial PGi, see below).

The locations of antidromically driven PGi cells are plotted in Fig. 4A. Note that the distribution of cells antidromically driven from LC in the ventrolateral medulla is similar to that of retrogradely labeled neurons (Fig. 4B) following a unilateral injection of WGA–HRP into LC, with the majority of cells projecting to LC detected by each method located in PGi caudal to the facial nucleus. In addition, it can be seen that spontaneously active neurons projecting to LC are located in the medial half of PGi, while non-spontaneous neurons are distributed more uniformly throughout the nucleus.

In the two halothane anesthetized animals, 3 of 9 cells were antidromically driven. These 3 cells were not spontaneously active and were driven at latencies (1.5, 7.2 and 9.5 ms) similar to other non-spontaneous antidromic cells.

Two additional non-spontaneous PGi neurons were antidromically activated from LC at latencies of 3.5 and 5.5 ms, but only with high stimulation intensities (>900 μA) and were therefore not included in the data analyses. We also found 9 PGi neurons to be synaptically activated from LC stimulation (mean threshold = 539 μA) at latencies ranging from 2 to 12 ms.

Sensory responses of PGi neurons. Previous studies in anesthetized rats reveal that LC neurons are activated by noxious sensory stimulation, such as tail pinch or electrical stimulation of the hindpaw[10,17]. We therefore investigated the possibility that PGi

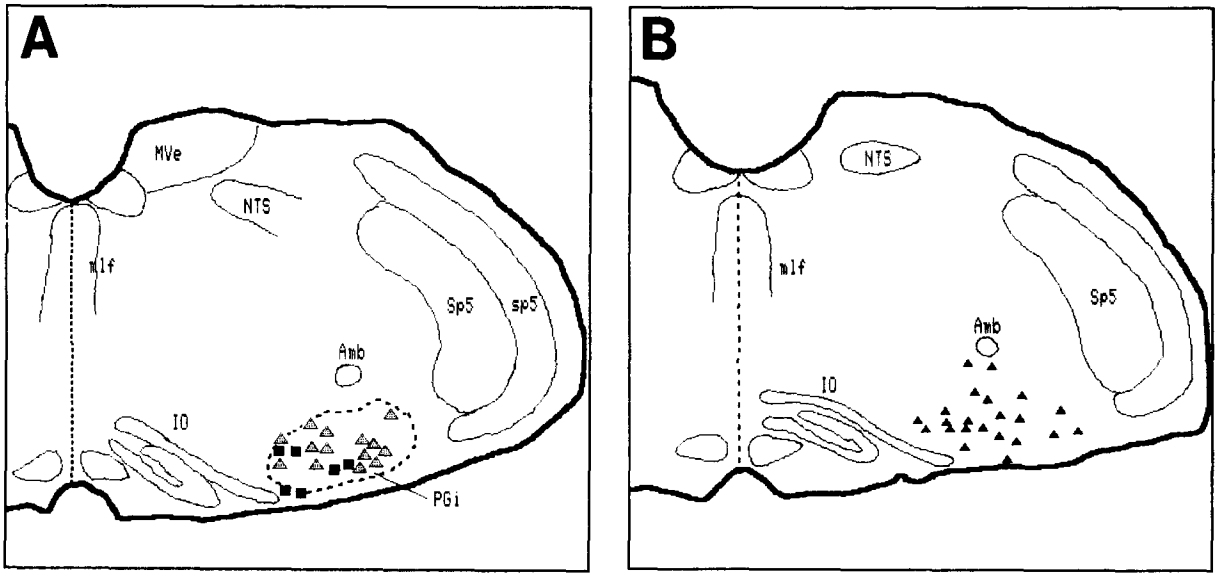


Fig. 4. A: video computer-aided reconstruction of a coronal section taken through the rostral medulla showing the locations of antidromically activated PGi neurons (composite figure from 7 rats). Note that spontaneously active cells (solid squares) are located in the medial PGi while non-spontaneous cells (triangles) are distributed more uniformly throughout PGi. Amb, nucleus ambiguous; IO, inferior olive; mlf, medial longitudinal fasciculus; MVe, medial vestibular nucleus; NTS, nucleus tractus solitarius; PGi, nucleus paragigantocellularis; Sp5, spinal nucleus of the Vth nerve:; sp5, spinal tract of the Vth nerve. B: computer-aided composite plot of retrogradely labeled neurons (solid triangles) in two 50-μm coronal sections taken through PGi following a unilateral injection of WGA–HRP into rat LC[6].

neurons may respond to similar stimuli, finding that many PGi neurons were synaptically activated by tail pinch or pressure applied to the scalp wound. Subcutaneous electrical stimulation (20–50 V) of the contralateral hindpaw (Fig. 5) or tail pinch robustly activated 22 of 33 PGi cells at latencies ranging from 8 to 35 ms, with single stimuli often yielding 2–3 spikes. The mean onset of latency for such activation was 11 ± 1.1 ms, and mean duration was 13 ± 1.5 ms (n = 17 cells). Five of 13 cells antidromically identified as LC afferents were synaptically activated by hindpaw stimulation. Four of these cells were non-spontaneous. In addition, several cells examined in PGi were activated by light strokes applied to the flank or back.

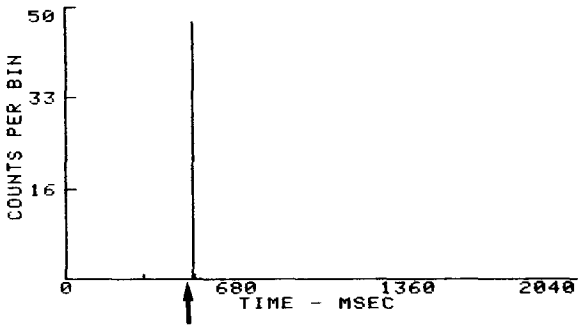


Fig. 5. Peri-stimulus time histogram generated during electrical stimulation of the hindpaw. Hindpaw stimulation (at arrow, 40 V) activates this PGi neuron beginning at 15 ms. Activity accumulated for 50 consecutive stimuli presented at 0.5 Hz. Bin width = 8 ms.

DISCUSSION

The present findings provide physiologic evidence for a substantial projection from the nucleus PGi to LC. Focal electrical stimulation of LC frequently antidromically activated PGi neurons (20 of 79 cells) at low stimulation intensities. In contrast, only 2 of 44 cells localized to LRN were antidromically driven from LC, and one of these was near the caudal border of PGi. These results indicate that LRN provides virtually no innervation of LC. Thus, the relative frequencies of antidromic activation in PGi and LRN from LC provide physiologic confirmation of our recent anatomic report[6] of a major projection to LC from PGi but not from LRN area.

Approximately 25% of PGi neurons were anti-

dromically activated from LC, indicating a substantial projection from this nucleus to LC. It should be noted however, that the majority of driven cells encountered were non-spontaneous, and it is possible that some non-spontaneous neurons that do not innervate LC were not detected on micropipette penetrations through PGi. Thus, the present results may overestimate the percentage of neurons in PGi that project to LC.

Several lines of evidence from the present and previous studies in our laboratory indicate that the antidromic driving observed here in PGi was a consequence of direct activation of PGi axons innervating LC. (1) The antidromic nature of driven spikes was verified in the present experiments by use of collision testing and high frequency activation. (2) Stimulation amplitudes were kept below the threshold for facial twitching; stimulation was therefore presumably confined to LC, avoiding activation of the trigeminal nerve nucleus immediately adjacent to LC or other nearby structures. (3) The anatomic distribution of antidromically activated PGi neurons coincides with PGi neurons retrogradely labeled from LC[6] (Fig. 4B). (4) Injections of WGA–HRP into PGi produce dense anterograde labeling in LC[6]. (5) Focal electrical stimulation of PGi synaptically activates LC neurons at latencies (mean onset = 11.7 ms[18]) compatible with the antidromic latencies observed here.

Several observations in this study indicate that there are two populations of PGi neurons projecting to LC. Non-spontaneous neurons, exhibiting small negative impulse waveforms, were driven at significantly shorter latencies (mean latency = 6.1 ms) than spontaneously active cells exhibiting large positive waveforms (mean latency = 17 ms). In addition, spontaneously active, antidromically driven PGi neurons were preferentially located in the medial half of PGi, while non-spontaneous LC-projecting neurons were distributed throughout PGi. Thus, these physiologically distinct populations of LC-projecting neurons also differ in their anatomic positions within PGi. It is noteworthy that in a study of PGi cytoarchitectonics, Andrezic et al.[1] observed that although PGi neurons are diverse in cellular morphology, large perikarya are distributed primarily in the caudal and medial PGi while smaller neurons comprise the majority of PGi cells and are distributed throughout the nucleus. Thus, large, medially located PGi neurons projecting to LC may primarily generate positive waveforms, spontaneous activity, and relatively slow impulse conduction speeds, while smaller more uniformly distributed PGi cells innervating LC may lack spontaneous activity (at least in the anesthetized animal) and yield negative spikes with more rapid conduction velocities. It is also interesting to note that 4 of the 5 LC-projecting neurons in PGi that responded to noxious stimuli were of the latter cell type.

The results of several immunocytochemical studies reveal a fairly direct fiber pathway from the ventrolateral medulla to LC[3,22]. With an estimated straight line distance of aproximately 4.0 mm from PGi to LC, the antidromic latencies obtained here translate to a mean impulse conduction velocity for LC-projecting PGi fibers of 0.9 ± 0.2 m/s (range = 0.2–2.7 m/s). Similar calculations for the latencies of spontaneous and non-spontaneous PGi cells projecting to LC yield mean conduction velocities of 0.5 and 1.1 m/s, respectively. Interestingly, previous work has also demonstrated two populations of spinally projecting PGi neurons differing in conduction velocities[9,20,32]; work by others[33] indicates that the slowly conducting spinal projections are likely to be non-myelinated, while the more rapidly conducting cells may contain a thin myelin sheath. Similarly, our conduction velocity data indicate that fibers of the slowly conducting spontaneously active LC afferents from PGi are probably non-myelinated, while fibers of the more rapidly conducting non-spontaneous group may be thinly myelinated. It is tempting to speculate that a portion of the slowly conducting PGi neurons observed here and those (putatively adrenergic[32]) reported by others represent an overlapping population of cells, so that some PGi neurons may project to both the spinal cord and LC. In view of findings that PGi neurons are found to innervate the preganglionic sympathetic column of the spinal cord[26,27,28,34], this possibility may shed light on the observation that LC neurons and sympathetic nerves exhibit similar activity profiles in some circumstances[14,15]. The possibility that at least some of the slowly conducting LC afferent neurons in PGi are adrenergic is also consistent with previous reports of an adrenergic innervation of LC[3,22], and recent findings that some PGi neurons retrogradely labeled from LC also stain for phenyl-N-methyl-transferase[21,29], a marker of adrenergic

cells.

Two populations of caudal ventrolateral medullary cells that differ in conduction velocity have also been found to innervate the paraventricular nucleus of the hypothalamus[25]. The majority (80%) of rapidly conducting hypothalamic-projecting neurons were non-spontaneous while most (85%) slow conducting cells were spontaneously active, resembling the present results for LC-projecting PGi neurons. Thus, physiologically similar neurons in the ventrolateral medulla may innervate the hypothalamus and LC.

Discharge of PGi cells has been reported to vary with blood pressure, respiratory and cardiac cycles, and pCO_2 (refs. 8, 9, 19). Thus, some properties of PGi cells determined here for anesthetized rats may differ from those in unanesthetized preparations. It is noteworthy however, that similar results were obtained in the present experiments using chloral hydrate or halothane anesthesia.

The finding that many PGi neurons were synaptically activated by noxious cutaneous stimulation or by electrical stimulation of the contralateral hindpaw is consistent with reports by others that PGi neurons respond to noxious stimuli[7,30]. Previous studies have also revealed activation of PGi neurons by non-noxious stimuli[7,23]. These observations, in light of inputs to PGi from sensory nuclei and the spinal cord[2], indicate that PGi may function, at least in part, to integrate polymodal sensory information. In this context, it is interesting to note that LC neurons are also activated by both noxious and non-noxious somatosensory stimuli[5,10] and that some PGi neurons identified as projecting to LC in the present study were excited by hindpaw stimulation. These findings provide indirect evidence that the PGi may participate in neural circuits by which sensory stimuli influence LC. This possibility is consistent with our recent report that focal electrical stimulation of PGi potently activates LC neurons synaptically at latencies (mean = 11.7 ms[18]) somewhat shorter than response onsets for LC neurons following external stimuli (18 ms for tone pips[5], and about 20 ms for hindpaw stimulation[10].

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