

## BRAIN AFFERENTS TO THE LATERAL CAUDAL VENTROLATERAL MEDULLA: A RETROGRADE AND ANTEROGRADE TRACING STUDY IN THE RAT

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**Abstract**—The ventrolateral medulla (VLM) modulates autonomic functions, motor reactions and pain responses. The lateralmost part of the caudal VLM (VLMlat) was recently shown to be the VLM area responsible for pain modulation. In the present study, the brain sources of VLMlat afferent fibers were determined by tract-tracing techniques. Following injection of cholera toxin subunit B into the VLMlat, retrogradely labeled neurons in the forebrain occurred at the somatosensory, insular, motor, limbic and infralimbic cortices, and at the central amygdaloid nucleus. Retrogradely labeled neurons in diencephalic regions were observed in the lateral hypothalamus, posterior hypothalamus and paraventricular nucleus. In the brainstem, retrograde labeling occurred at the periaqueductal gray, red nucleus, parabrachial area, nucleus raphe magnus, nucleus tractus solitarii, lateral reticular nucleus and dorsal and ventral medullary reticular formation. In the cerebellum, retrogradely labeled neurons occurred at the lateral nucleus. Following injections of the anterograde tracer biotinylated dextran amine (BDA) into the lateral hypothalamus or paraventricular nucleus, anterogradely labeled fibers were mainly observed in the VLMlat. Injections of BDA into the periaqueductal gray, red nucleus or lateral nucleus of the cerebellum resulted in anterograde labeling in the VLMlat and lateral reticular nucleus.

The present study gives an account of the brain regions

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**Abbreviations:** ABC, avidin–biotin complex; BDA, biotinylated dextran amine; BST, bed nucleus of the stria terminalis; CeA, central amygdaloid nucleus; CTb, cholera toxin subunit B; CVLM, caudal ventrolateral medulla; DAB, diaminobenzidine tetrahydrochloride; DRt, dorsal reticular nucleus; IC, insular cortex; IL, infralimbic cortex; IO, inferior olive; KF, Kölliker-Fuse nucleus; Lat, lateral (dentate) cerebellar nucleus; LC, locus coeruleus; LH, lateral hypothalamic area; LRt, lateral reticular nucleus; M<sub>1</sub>, primary motor cortex; M<sub>2</sub>, secondary motor cortex; Pa, paraventricular hypothalamic nucleus; PAG, lateral periaqueductal gray; PB, phosphate buffer; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline containing 0.5% Triton X-100; PRL, prelimbic cortex; R, red nucleus; RMg, raphe magnus nucleus; RVLM, rostral ventrolateral medulla; SO, superior olive; Sol, nucleus tractus solitarii; Sp<sub>5</sub>C, spinal trigeminal nucleus, pars caudalis, VLM, caudal ventrolateral medulla; VLMlat, lateralmost part of the caudal ventrolateral medulla; VLPAG, ventrolateral periaqueductal gray; VRt, ventral reticular nucleus.

putatively involved in triggering the modulatory actions elicited from the VLMlat. These include areas committed to somatosensory processing, autonomic control, somatic and visceral motor activity and affective reactions. The findings suggest that the VLMlat may play a major homeostatic role in the integration of nociception with other brain functions. © 2003 IBRO. Published by Elsevier Science Ltd. All rights reserved.

**Key words:** pain modulation, motor control, cardiovascular regulation, cholera toxin subunit B, biotinylated dextran amine.

The ventrolateral medulla (VLM) is a functionally complex and heterogeneous brain area. The regulation of cardiovascular and respiratory functions is among the main autonomic functions performed by the VLM. The rostral VLM (RVLM) exerts vasopression through bulbospinal adrenergic neurons of the C<sub>1</sub> adrenergic cell group, present in the RVLM (Willette et al., 1983; Kalia et al., 1985; reviewed by Chalmers and Pilowski, 1991). The caudal VLM (CVLM) exerts vasodepression and contains the A<sub>1</sub> noradrenergic cell group (Day et al., 1983; Willette et al., 1983; Kalia et al., 1985; Murugaian et al., 1989; reviewed by Chalmers and Pilowski, 1991). The RVLM also contains most of the VLM respiratory neurons (Saper, 1995). The VLM participates in motor control through cerebellar and rubral connections of the lateral reticular nucleus (LRt; Alstermark et al., 1981; Clendenin et al., 1974; Ekerot, 1989), located at the CVLM. Pain control from the VLM is elicited from the CVLM, which is one important component of the endogenous pain modulatory system (Gebhart and Ossipov, 1986; Sotgiu, 1986; Janss and Gebhart, 1987, 1988; Liu and Zhao, 1992; reviewed by Tavares and Lima, 2002). The analgesia produced by CVLM stimulation is more profound and long-lasting than that obtained from other pain control centers (Satoh et al., 1983; Jensen and Yaksh, 1984; Carstens and Watkins, 1986; Jones and Gebhart, 1986a,b; Ness and Gebhart, 1987). At the CVLM, the reticular formation located between the LRt and the spinal trigeminal nucleus, pars caudalis (Sp<sub>5</sub>C), is the area specially devoted to pain modulation (reviewed by Tavares and Lima, 2002). This area of the CVLM, which has been named lateralmost part of the caudal ventrolateral medulla (VLMlat), is located ventrolaterally to the A<sub>1</sub> noradrenergic cell group (Tavares et al., 1996, 1997a) and projects exclusively to spinal cord layers involved in the transmission of nociceptive information (Tavares and Lima, 1994; Tavares et al., 1998; reviewed by Tavares and Lima, 2002). Furthermore, the electrical threshold necessary to produce

analgesia from the VLMIat is lower than in the other CVLM areas (Gebhart and Ossipov, 1986). Finally, the VLMIat contains neurons that are the source of a dysynaptic pathway relayed in the noradrenergic pontine A<sub>5</sub> area, which conveys the spinal  $\alpha_2$ -adrenoreceptor-mediated analgesia elicited from the VLM (Tavares et al., 1996, 1997a).

Integration of autonomic functions, motor control and pain modulation is crucial to body homeostasis, namely in life-threatening conditions (Spyer, 1989; Lovick, 1993; Harris, 1996). All those functions are played by the VLM, although somewhat segregated within this region. For example, the VLMIat, which is the pain region of the CVLM, is lateral to the cardiovascular region described by Blessing and Li (1989). It is possible that the VLM participates in the overall integration of inputs triggered in several areas of the CNS. In order to characterize the input arriving to the VLMIat it is important to map the afferents to this region. However, CNS projections to the CVLM have not been described as part of the thorough studies of projections from the spinal cord (Menétrey et al., 1983; Lima and Coimbra, 1991; Lima et al., 1991; Rajakumar et al., 1992; Ruigrok and Cella, 1995; Koekoek and Ruigrok, 1995). This study describes the brain regions that project to the VLMIat in the rat by retrograde tracing with cholera toxin subunit B (CTb) complemented with anterograde tracing with biotinylated dextran amine (BDA).

## EXPERIMENTAL PROCEDURES

Male Wistar rats from the Gulbenkian Institute of Science (Portugal), 285–305 g in weight, were used. The experiments followed the regulations of local authorities for handling laboratory animals and the European Communities Council Directive 86/609/EEC. All efforts were made to minimize the numbers of animals used in the experiments and to avoid suffering.

## Retrograde tracing studies

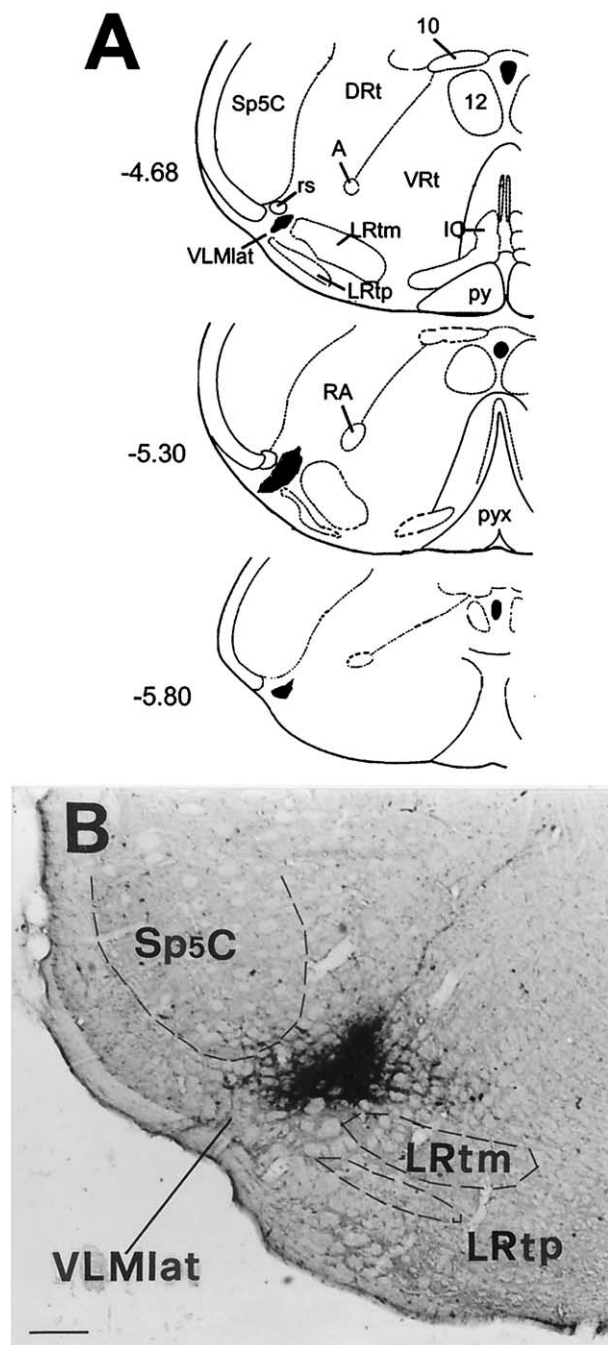
Fourteen rats were anesthetized with a gaseous mixture of halothane (4% for induction and 1.5–2% for maintenance), N<sub>2</sub>O (66%) and O<sub>2</sub> (34%). Seven animals were pressure injected, using a 1  $\mu$ l Hamilton syringe, with 0.3  $\mu$ l of a 1.5% CTb solution (List Biological Laboratories, Campbell, CA, USA), in the left VLMIat, according to the stereotaxic coordinates of Paxinos and Watson (1998). Two rats were iontophoretically injected in the same region with 1% of a low-salt CTb solution (List Biological Laboratories), using 25–30  $\mu$ m glass micropipettes and a 2  $\mu$ A continuous current, over 10 min. The remaining five animals were used for control injections, in areas surrounding the VLMIat. For this purpose the rats were pressure injected in the Sp<sub>5</sub>C ( $n=3$ ; 0.5  $\mu$ l) and LRT ( $n=2$ ; 0.4  $\mu$ l) with a CTb solution prepared as above.

After completion of the injections, Hamilton syringes or micropipettes were left *in situ* for 10–15 min, before being slowly retracted. Five days later, the animals were reanaesthetized with chloral hydrate (0.35 g/kg body weight) and perfused through the ascending aorta with 1000 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains and part of spinal segment C<sub>1</sub> were removed, immersed in fixative for 4 h and in 30% sucrose in PB, for 2 days at 4 °C. Before sectioning, the caudal part of the brains was trimmed at the level of the pyramidal decussation (about 0.8 mm caudal to the obex; Kalia and Fuxe, 1985).

Coronal sections were cut in a freezing microtome at 40  $\mu$ m and serially collected in 0.1 M phosphate-buffered saline (PBS) and two adjacent sections in every three were immunostained for CTb. Sections were washed in PBS containing 0.5% Triton X-100 (PBST) and incubated for 2–4 days, at 4 °C, in an anti-CTb antibody raised in goat (List Biological Laboratories), diluted at 1:40,000 in PBST. After repeated washings, the sections were incubated in a biotinylated horse anti-goat antiserum followed by an avidin–biotin complex reagent (ABC; Vector Laboratories (UK), Burlingame, CA, USA, both for 1 h, at 1:200. After a 10-min washing in 0.05 M Tris-buffered saline, pH 7.6, sections were incubated for 10–15 min with 0.1% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.012% H<sub>2</sub>O<sub>2</sub>, diluted in the same buffer.

### Abbreviations used in the figures

3	oculomotor nucleus	LRtp	lateral reticular nucleus, parvicellular part
7	facial nucleus	ml	medial lemniscus
10	dorsal motor nucleus of vagus	MPA	medial preoptic area
12	hypoglossal nucleus	MPB	medial parabrachial nucleus
3V	3 <sup>rd</sup> ventricle	opt	optic tract
4V	4 <sup>th</sup> ventricle	PeF	perifornical nucleus
A	ambiguus nucleus	PFI	paraflocculus
A <sub>5</sub>	A <sub>5</sub> noradrenergic cell group	PH	posterior hypothalamic area
ac	anterior commissure	PR	prerubral field
Acb	nucleus accumbens	Py	pyramidal tract
AP	area postrema	Pyx	pyramidal decussation
APT	anterior pretectal nucleus	RCh	retrochiasmatic area
Aq	aqueduct Sylvius	scp	superior cerebellar peduncle
cc	central canal	SI	substantia innominata
Cu	cuneate nucleus	SNR	substantia nigra, reticular part
DEn	dorsal endopiriform nucleus	Sp <sub>5</sub> O	spinal trigeminal nucleus, pars oralis
DLPAG	dorsolateral periaqueductal gray	SubC	subcoeruleus nucleus
DpMe	deep mesencephalic nucleus	TC	tuber cinereum area
DR	dorsal raphe nucleus	VA	ventral anterior thalamic nucleus
EW	Edinger-Westphal nucleus	Ve	vestibular nucleus
f	fornix	VL	ventrolateral thalamic nucleus
ic	internal capsule	VP	ventral pallidum
icp	inferior cerebellar peduncle	VPL	ventral posterolateral thalamic nucleus
LPB	lateral parabrachial nucleus	VTRZ	visual tegmental relay zone
LPO	lateral preoptic area	ZI	zona incerta
LRtm	lateral reticular nucleus, magnocellular part		



**Fig. 1.** CTb injection sites in the VLMIat. In A, diagrams depicting the injection site in animal 2356, in three medullary sections obtained at the rostrocaudal level indicated by the distance (in mm) from the interaural line. The injection sites include the central core (black) and the peripheral halo (gray). In B, photomicrograph of an injection site in the VLMIat at the level  $-4.68$  mm, in animal 1346. Scale bar =  $0.16$  mm.

After washing in PBS, the first set of sections of the two adjacent sections out of three taken from the section series was mounted on gelatin-coated slides, cleared in xylene and coverslipped with Eukitt. In order to delimitate brain nuclei, the additional set of sections was counterstained with formol–Thionin (Donovick, 1974) and camera lucida drawings were performed according to Paxinos and Watson (1998). Retrogradely labeled neurons that

occurred in five sections randomly taken from the rostrocaudal levels indicated in Fig. 2C, were plotted in the respective drawings. For quantitative analysis, all the retrogradely labeled neurons occurring in the brain nuclei identified in Thionin-stained sections were counted. The seven animals with pressure injections in the VLMIat were considered for quantitative analysis by counting the retrogradely labeled neurons plotted in camera lucida drawings.

### Anterograde tracing experiments

Eleven rats were anesthetized as above and iontophoretically injected with a 10% BDA solution (mol. wt: 10 kDa; Molecular Probes Inc., Leiden, The Netherlands) in 0.1 M PB, pH 7.2, through 25–30  $\mu\text{m}$ -diameter-tip glass micropipettes using a positive 2.5  $\mu\text{A}$  DC current for 20–30 min. The left part of the lateral hypothalamic area (LH;  $n=3$ ), paraventricular nucleus (Pa;  $n=2$ ), red nucleus (R;  $n=2$ ), ventrolateral periaqueductal gray (VLPAG;  $n=2$ ) and lateral nucleus of the cerebellum (Lat;  $n=2$ ), were injected following the stereotaxic coordinates of Paxinos and Watson (1998).

The rats were reanesthetized and perfused as described in the retrograde tracing experiments. Serial 50  $\mu\text{m}$ -thick frozen sections passing through the injection sites and from the caudal medulla oblongata were incubated for 1 h with ABC, as above. Bound peroxidase was revealed using DAB as above and, after repeated washing with PBS, one set of reacted sections was mounted on gelatin-coated slides while the other set was counterstained with formol–Thionin (Donovick, 1974).

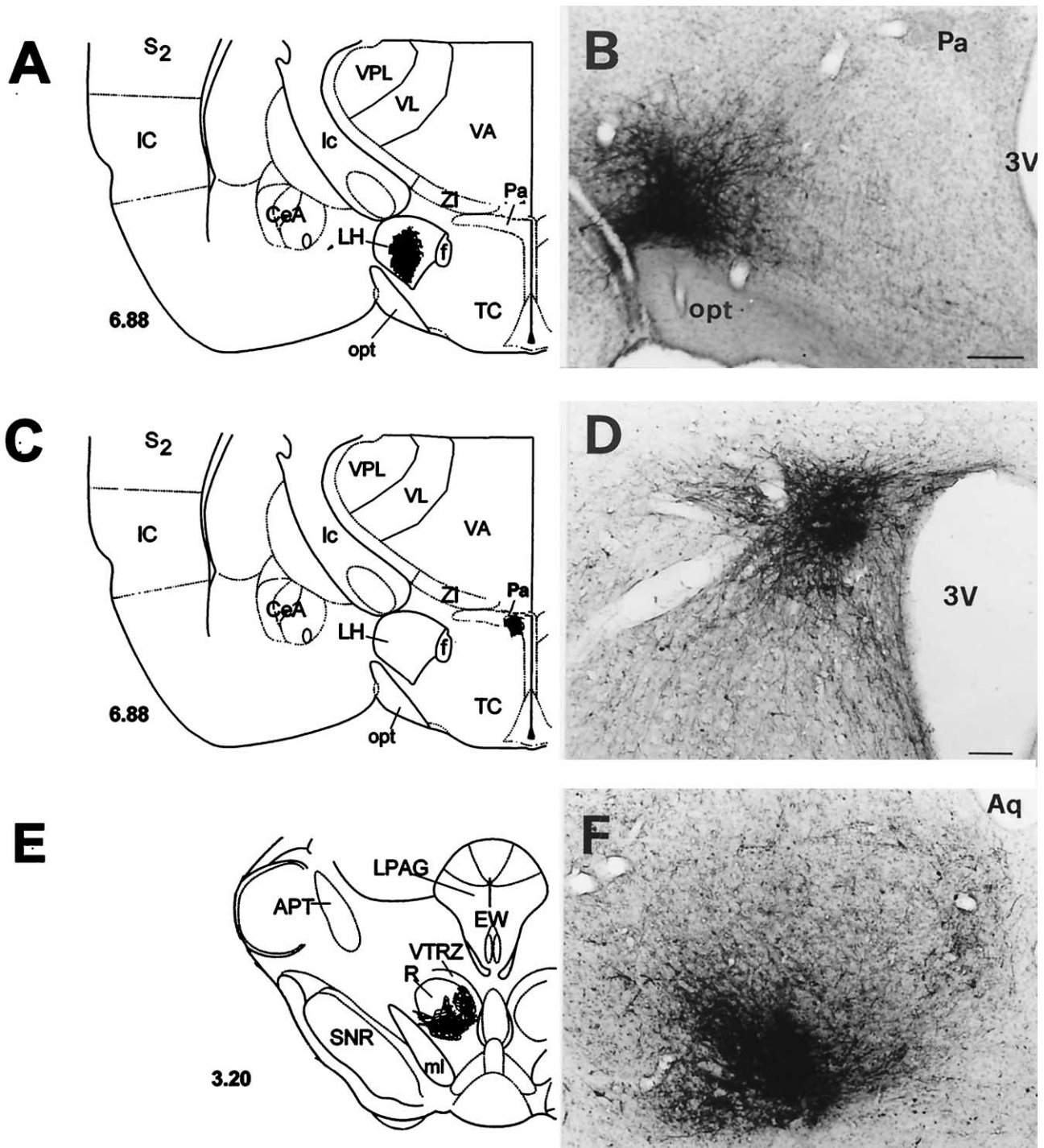
The distribution of anterogradely labeled fibers at the CVLM was established using a procedure similar to that described for CTb, but no quantification was performed. Five well-stained sections taken from two rostrocaudal levels of the medulla oblongata (4.8 and 5.8 mm caudal to the interaural line) were used to obtain camera lucida drawings depicting anterogradely labeled fibers. The distinct CVLM subareas were identified according to previous cytoarchitectonic studies (Kalia and Fuxe, 1985; Kapogianis et al., 1982; Paxinos and Watson, 1998).

## RESULTS

### Injection sites

CTb injection sites consisted of a homogeneous, compact core surrounded by a halo in which dark areas intermingled with lighter zones (Fig. 1). A few retrogradely labeled cells were observed around the peripheral halo probably due to uptake from the more central areas. In accordance with previous studies (Ericson and Blomqvist 1988; Lima et al., 1991), only the central core and the peripheral halo were included in the injection sites. In the animals considered for quantitative analysis of retrograde labeling, the injection sites were restricted to the VLMIat, between the levels of the area postrema and pyramidal decussation (Fig. 1A). Control injections in the Sp<sub>5</sub>C were located in the ventral third of the nucleus while injections in the LRt encompassed mainly its magnocellular component.

BDA injection sites presented a granular core of densely packed perikarya surrounded by a narrow rim formed by scarcely labeled neurons (Fig. 2). More peripherally there was a region of densely labeled fibers, probably indicating uptake from the more central areas. Only the central region and the rim were included in the



**Fig. 2.** Diagrams and photomicrographs of BDA injection sites in the LH (A, B), Pa (C, D), R (E, F), VLPAG (G, H) and Lat (I, J). Panels B and H and figures D, F and J are at the same magnification. Scale bars=0.25 mm.

injection site (Veenmam et al., 1992; Wouterlood and Jorritsma-Byham, 1993). BDA injection sites in the LH were centered in the median half of the nucleus (Fig. 2A, B). Injections in the Pa encompassed mainly the medial parvocellular component of the nucleus (Fig. 2C, D), reaching occasionally the lateral magnocellular part. In-

jections in the R encompassed both the magno- and parvocellular parts of the nucleus (Fig. 2E, F). Injections in the VLPAG were located on its median portion (Fig. 2G, H). Cerebellar injections were located in the Lat extending ventrally to reach the borders of the inferior cerebellar peduncle (icp; Fig. 2I, J).

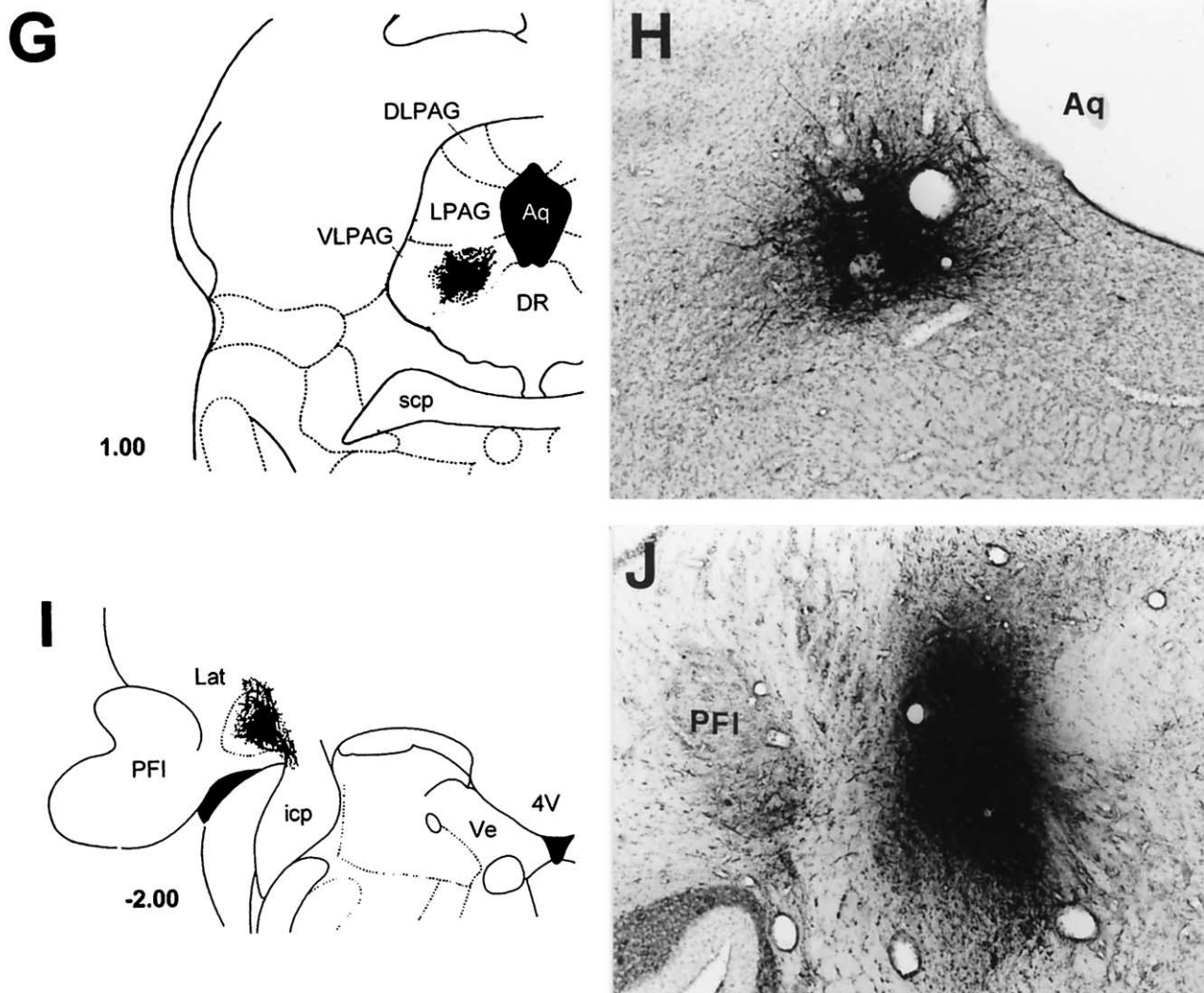


Fig. 2. (G–J).

### Retrograde labeling with CTb

*Injections in the VLMIat.* After injections in the VLMIat, retrogradely labeled neurons were observed in several areas along the rostrocaudal extension of the brain (Figs. 3 and 4; Table 1). Similar patterns of retrograde labeling were observed after pressure and iontophoretic deliveries, but counting was only performed in the former injection type (Table 1).

### Forebrain

At cortical regions, retrogradely labeled neurons largely prevailed at the primary and secondary somatosensory cortex, with a contralateral predominance (Figs. 3B–D and 4A). Retrogradely labeled neurons also occurred in the insular cortex (IC) and, more moderately, in the primary and secondary motor cortex ( $M_1$  and  $M_2$ ), mainly contralaterally (Fig. 3A–D). In four animals (1377, 2262, 2263 and 2356), some CTb labeled neurons occurred bilaterally at

the prelimbic (PRL)/infralimbic (IL) cortex (Fig. 3A, B). Other forebrain regions with retrogradely labeled neurons included the central amygdaloid nucleus (CeA; Figs. 3D and 4B), bed nucleus of the stria terminalis (BST; Fig. 3C) and dorsal endopiriform nucleus (Fig. 3B), with an ipsilateral prevalence.

### Diencephalon

At the diencephalon, the most prominent projection was originated from the hypothalamus, mainly at the LH and Pa, with an ipsilateral prevalence (Figs. 3D, 4C). Lower numbers of labeled neurons were observed at the posterior hypothalamus, preoptic region of the hypothalamus, tuber cinereum area, perifornical region, zona incerta and retrochiasmatic area, mainly ipsilaterally (Fig. 3C). In some animals scarce retrograde labeling was observed in the region of the ventral pallidum and substantia innominata and in the nucleus accumbens, mainly ipsilaterally (Fig. 3C).

### Mesencephalon

At the mesencephalic level, the most prominent projection was originated from the periaqueductal gray (PAG), mainly from the VLPAG, bilaterally (Figs. 3E, 4D). In some animals, retrogradely labeled cells were observed at the region of the Edinger-Westphal and dorsal raphe nuclei in the R and oculomotor nucleus (3), with a contralateral prevalence, and bilaterally in the deep mesencephalic nucleus (Figs. 3E, 4D).

### Pons and cerebellum

The parabrachial complex was the major source of pontine projections to the VLMIat. High numbers of neurons were observed at the lateral parabrachial nucleus, mainly ipsilaterally (Figs. 3F, 4E). Some retrogradely labeled neurons were observed at the medial parabrachial and Kölliker-Fuse (KF) nuclei, mainly ipsilaterally (Fig. 3F). The region of the A<sub>5</sub> noradrenergic cell group also presented intense bilateral retrograde labeling (Fig. 3F). Some neurons occurred bilaterally in the coerulear region namely in the locus coeruleus (LC) and nucleus subcoeruleus (Fig. 3F and 4E). More moderate bilateral labeling was observed in the region of the superior olive (SO; Fig. 3F).

In four animals (1378, 1408, 2262 and 2263) retrogradely labeled neurons occurred at the cerebellum, namely in the Lat, with an ipsilateral prevalence (Fig. 3G).

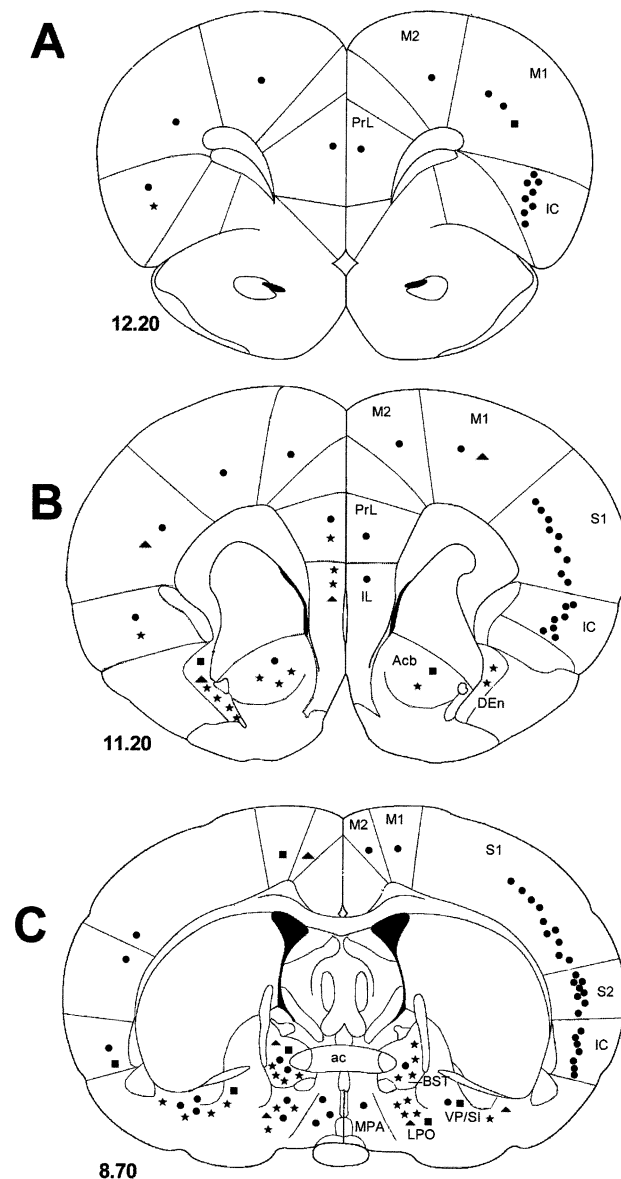
### Medulla oblongata

At the medulla oblongata, very intense retrograde labeling was observed at the superficial layers of the Sp<sub>5</sub> mainly ipsilaterally, and at the nucleus tractus solitarii (Sol; Figs. 3H, I, 4F). Moderate retrograde labeling was observed at the nucleus raphe magnus (RMg; Fig. 3F, G), with an ipsilateral prevalence. The dorsal (DRt) and ventral (VRt) reticular nuclei contained numerous retrogradely labeled neurons, bilaterally (Figs. 3H, I, 4F). Bilateral labeling was observed at the RVLM and at the magnocellular part of the LRt (Fig. 3H, I). A considerable amount of retrogradely labeled neurons occurred at the contralateral VLMIat (Fig. 3H, I). A few neurons were observed at the area postrema and, contralaterally, at the inferior olive (IO).

### Control injections

Following injections in the Sp<sub>5</sub>C, retrogradely labeled neurons in the forebrain occurred at the somatosensory cortex, contralaterally, and CeA, bilaterally. In the diencephalon, the most important projections occurred from the thalamus namely from the parafascicular thalamic nucleus and ventromedial thalamic nucleus, both bilaterally. Labeled brainstem regions included the PAG, namely its dorsolateral part and the parabrachial complex, both bilaterally, and all the rostrocaudal components of the trigeminal complex, mainly ipsilaterally.

After injections in the LRt, retrogradely labeled neurons occurred at the M<sub>1</sub>/M<sub>2</sub> regions and PRL/IL area. A very intense labeling was observed at the globus pallidus, substantia nigra, R and Lat, SO, DRt, Sol and IO.



**Fig. 3.** Diagrams illustrating the distribution of retrogradely labeled neurons in the brain after CTb injections in the VLMIat (in animal 2262). Each drawing was taken from representative rostrocaudal levels, indicated by the distance (in mm) from the interaural line. Symbols represent 20 (●), 10 (■), five (▲) or one (\*) neuron. Left side is ipsilateral to the injection site.

### Anterograde labeling with BDA

Following injections in the LH, strong anterograde labeling was observed bilaterally in the VLMIat (Fig. 5A, B) extending dorsomedially to the region of the ipsilateral A<sub>1</sub> noradrenergic cell group (Fig. 5A). Injections in the Pa produced intense labeling of the VLMIat, mainly ipsilaterally (Fig. 5C), which reached occasionally the A<sub>1</sub> region. Injections in the R produced contralateral labeling mainly in the lateral LRt and VLMIat (Fig. 5D). After VLPAG injections, labeled fibers occupied mainly the

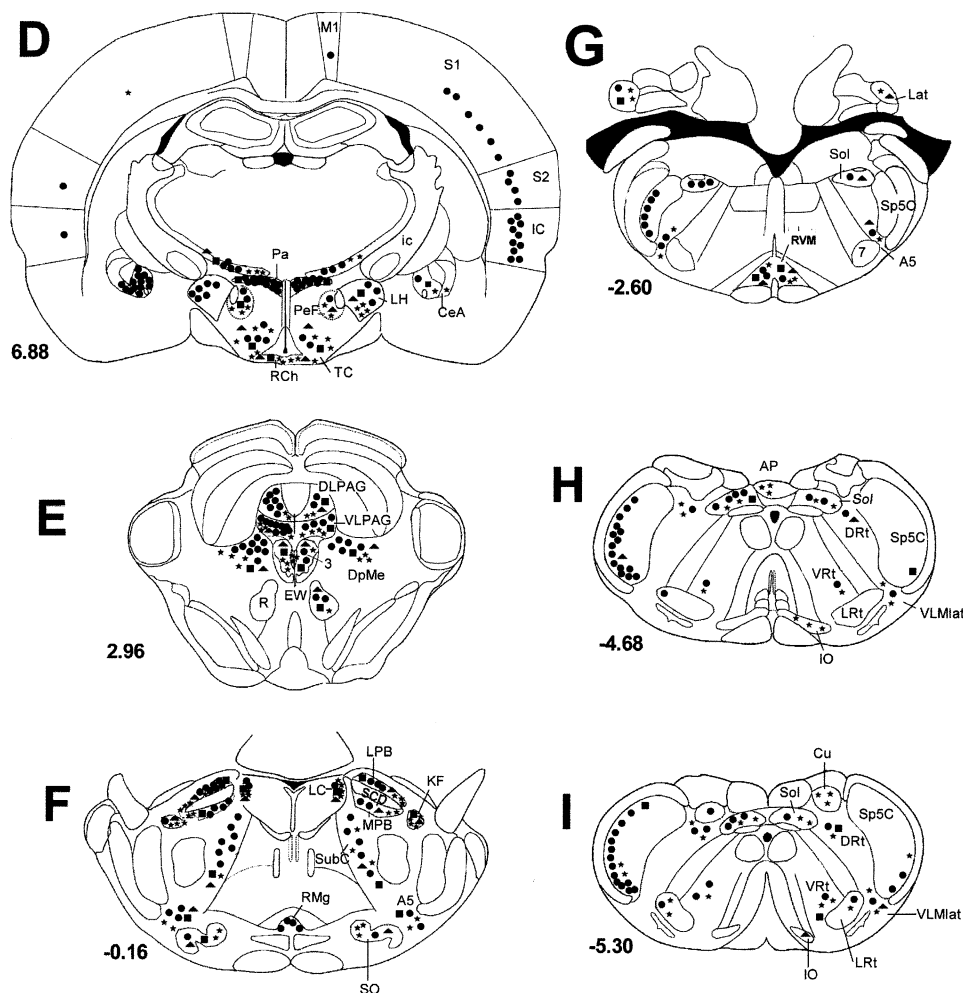


Fig. 3. (D–I).

VLMIat, bilaterally (Fig. 5E). A few occurred at the lateral LRt, with a slight ipsilateral prevalence. Cerebellar injections in the Lat resulted in anterograde labeling in the LRt and VLMIat, mainly ipsilaterally and, occasionally, in the A<sub>1</sub> region (Fig. 5F).

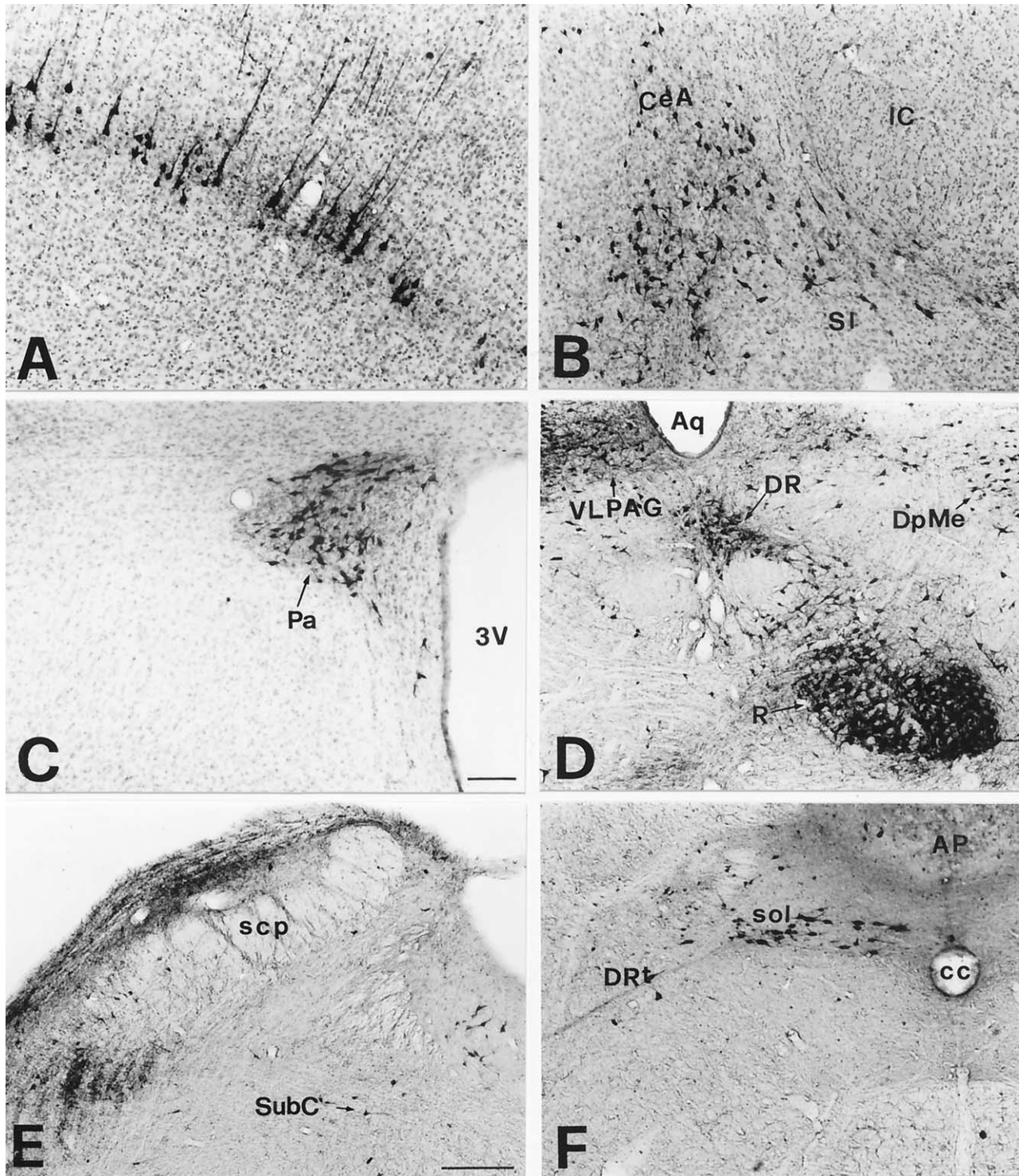
## DISCUSSION

### Technical considerations

The present study establishes the brain afferents to the VLMIat by the use of retrograde and anterograde tracing with, respectively, CTb and BDA. CTb was chosen because it produces intense retrograde labeling from relatively small injection sites (Ericson and Blomqvist, 1988; Lima and Coimbra, 1991; Lima et al., 1991; Tavares and Lima, 1994), which were here confined to the VLMIat. The small size of the CTb injection sites probably accounted to some inter-animal variability, namely in what concerns the lack of labeling of brain nuclei detected in some animals. Similar inter-animal variation was detected after small CTb injections in other CNS regions and was ascribed to unequal filling of territories

in such injection sites (Ericson and Blomqvist, 1988; Lima et al., 1991; Tavares and Lima, 1994). Retrograde labeling after injections in the VLMIat was probably produced exclusively by transport from that region since the pattern of labeling was different from those obtained after control injections in the surrounding regions. Along with negative outcomes of neuronal labeling due to CTb uptake by passing fibers (Lima et al., 1991; Tavares and Lima, 1994), some positive findings were reported (Luppi et al., 1990). It is, however, unlikely that fibers coursing in the VLMIat accounted for the neuronal labeling since these axons belong mainly to spinofugal pathways (Snyder et al., 1978; Yamada and Otani, 1978; Yamada et al., 1991). As to the rubrospinal tract, which courses laterally to the VLMIat (cf. Fig. 1A and Paxinos and Watson, 1998), it was probably not encompassed by the bulk of the injection sites. In agreement, rubral labeling was also demonstrated after CTb iontophoresis in the VLMIat, an injection procedure that prevents uptake by passing fibers (Luppi et al., 1990) and anterograde labeling was observed in the VLMIat after BDA injections in the R. These BDA tracing experiments,





**Fig. 4.** Photomicrographs depicting retrogradely labeled cells in the secondary somatosensory cortex (A), CeA/SI (B), Pa (C), R, DR and VLPAG (D), PBN region (E) and dorsal medulla oblongata (F) following CTb injections in the VLMIat. Panels A and D are contralateral to the injection site. Scale bars=150  $\mu$ m. Panels A–D and F are at the same magnification.

performed with the main purpose of clarifying the precise area of termination of some VLMIat afferent fibers were, therefore, a valuable complement of the retrograde tracing studies. The nuclei injected with BDA presented intense

retrograde labeling and were further selected based on their well-established involvement in the control of autonomic parameters (LH and Pa), nociception (PAG) and motor reactions (R and Lat; Paxinos, 1995).



**Table 1.** Numbers of neurons retrogradely labeled in the ipsilateral (ipsi) and contralateral (cont) forebrain (Foreb.), diencephalon (Dience.), mesencephalon (Mesence.), pons, cerebellum (Cereb.) and medulla oblongata (Med. obl.) after CTb injections in the VLMIat

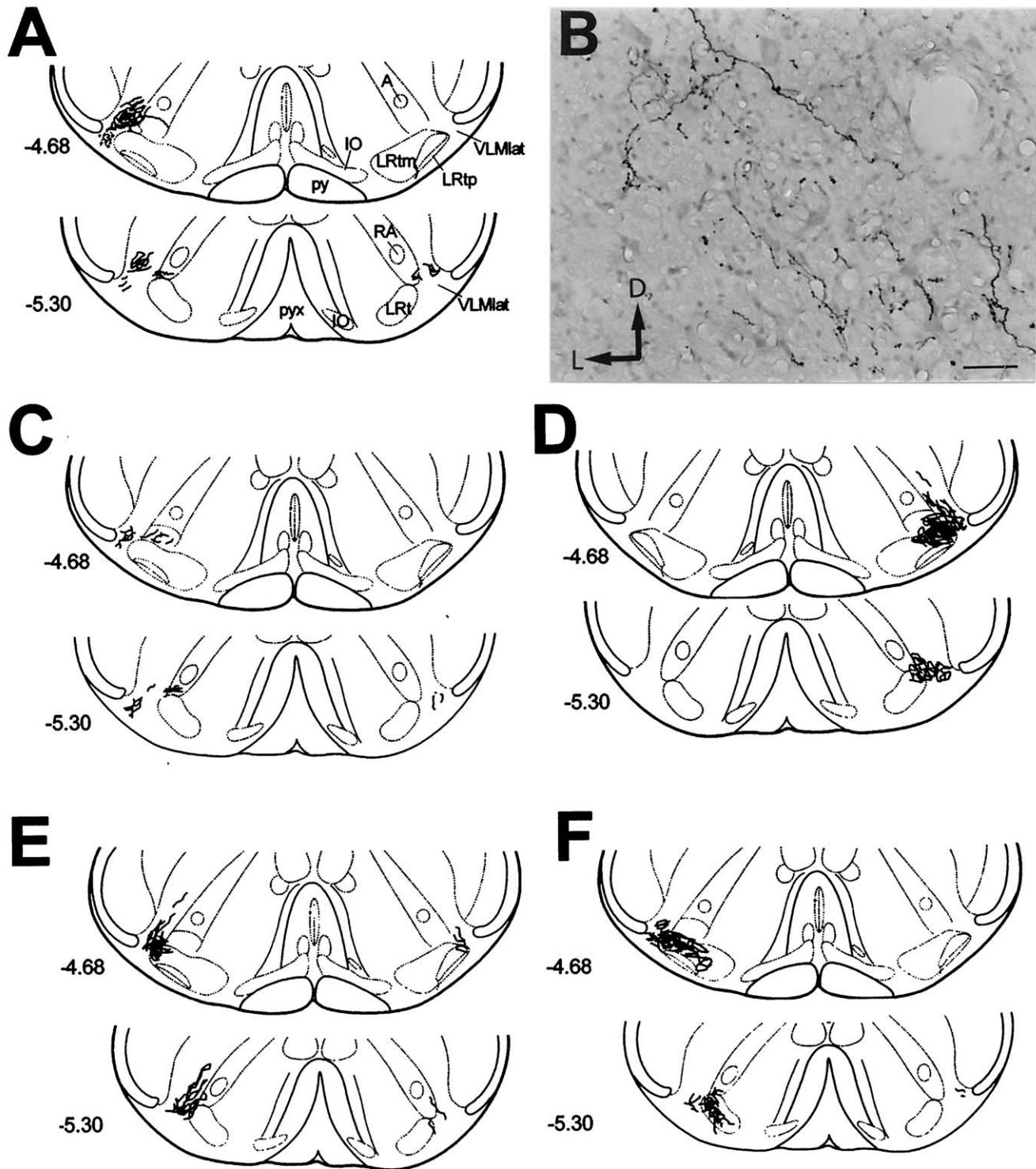
	1346		1377		1378		1408		2262		2263		2356	
	ipsi	cont	ipsi	cont	ipsi	cont	ipsi	cont	ipsi	cont	ipsi	cont	ipsi	cont
<b>Foreb.</b>														
M <sub>1</sub> /M <sub>2</sub>	46	48	48	97	3	24	36	96	95	175	256	484	0	0
S <sub>1</sub> /S <sub>2</sub>	76	659	65	910	54	160	32	780	85	740	232	1340	0	0
IC	78	191	51	413	15	169	91	600	92	640	150	695	64	189
PrL/IL	0	0	2	5	0	0	0	0	48	60	185	207	12	34
DEn	2	0	2	1	2	1	8	1	19	2	54	6	7	2
CeA	0	0	2	2	32	0	126	5	260	32	248	16	0	0
BST	0	0	18	6	12	0	12	0	59	24	107	12	14	6
<b>Dience.</b>														
VP/SI	0	0	51	22	0	0	0	8	74	36	65	21	0	0
AcB	0	0	9	2	7	1	0	0	23	11	17	8	0	0
MPA	0	0	8	2	1	0	8	2	60	20	62	21	0	0
LPO	0	0	12	4	2	0	12	0	48	19	52	18	0	0
TC	15	19	26	7	15	3	21	17	98	58	116	44	2	6
RCh	3	2	5	2	2	0	24	2	19	8	14	8	3	2
Pa	58	37	90	29	67	12	112	61	250	190	280	182	99	38
LH	325	78	74	24	40	21	150	67	120	79	159	92	110	82
ZI	78	32	56	11	12	10	59	35	98	72	31	23	45	26
PeF	26	36	4	2	4	4	15	10	58	27	71	23	36	15
PH	56	25	4	2	6	2	9	6	58	16	49	28	11	5
<b>Mesence.</b>														
DLPAG	10	2	20	8	5	2	29	8	142	57	92	36	4	2
VLPAG	11	21	85	52	43	6	84	24	189	94	210	96	24	13
DpMe	0	0	54	28	19	13	68	45	197	118	183	109	5	3
R	0	0	0	190	0	31	0	55	0	56	0	175	0	52
3	0	0	9	29	13	18	3	22	18	56	19	68	35	76
EW/ DR	0	0	3	1	2	1	5	2	2	6	5	6	4	3
VTRZ	0	1	0	32	1	7	1	7	18	27	12	24	0	0
<b>Pons</b>														
LPB	23	5	57	28	29	6	98	27	159	78	144	60	0	0
MPB	5	3	6	4	3	3	20	7	92	47	98	52	0	0
KF	14	2	43	8	17	4	18	11	28	19	56	40	0	0
A <sub>5</sub>	32	8	51	12	48	9	72	52	119	78	105	58	24	16
LC	2	1	14	8	9	5	16	15	58	39	32	28	28	22
SO	0	0	0	0	0	0	0	0	37	28	32	35	58	36
SubC	11	4	47	15	15	5	28	26	156	118	22	63	10	6
<b>Cereb.</b>														
Lat	0	0	0	0	10	3	18	9	32	6	27	5	0	0
<b>Med. obl.</b>														
AP	0	0	2	0	2	0	2	0	3	0	5	0	6	0
Sol	0	0	125	46	112	22	196	82	214	89	237	96	420	38
Sp <sub>5</sub> C	0	0	698	26	344	18	654	47	697	76	897	94	765	58
DRt	42	38	52	30	35	11	76	38	64	56	65	58	82	44
Cu	6	0	6	0	5	0	14	0	20	3	23	2	15	3
RMg	0	0	16	10	17	4	42	14	96	77	93	81	48	12
IO	0	0	0	14	0	0	0	22	0	8	0	14	0	138
RVLM	0	0	0	0	15	11	32	30	63	52	75	61	42	18
VRt	0	0	65	34	13	8	62	40	62	54	73	59	58	52
LRt	0	0	7	4	0	0	56	51	43	21	51	23	35	12
VLMIat	—	0	—	24	—	20	—	49	—	49	—	74	—	85

Depending on the rostrocaudal extension of each nucleus, four to eight sections were used to determine the number of retrogradely labelled neurons. For abbreviations see list.

### Somatosensory and autonomic afferents

The most prominent projections to the VLMIat were originated from areas related to sensory functions. To the best of our knowledge, projections from the somatosensory

cortex to the VLMIat were described for the first time in the present study. The somatosensory and insular cortices were shown to participate in pain control since analgesia is produced upon local instillation of morphine and spinal



**Fig. 5.** Diagrams depicting anterograde labeling at the VLM following BDA injections in the LH (A, B), Pa (C), R (D), VLPAG (E) and Lat (F). Each triangle in A corresponds to one noradrenergic neuron of the A<sub>1</sub> cell group, as seen in an immunostaining for dopamine-β-hydroxylase (Tavares et al., 1996, 1997a). In B, a photomicrograph of labeled fibers in the VLMlat following an injection in the LH. Arrows in the left corner of B indicate dorsal (D) and lateral (L) directions. Scale bar=20 μm.

nociceptive neurons are inhibited by electrical stimulation (Soto-Moyano et al., 1988; Burkey et al., 1996). However, the somatosensory and insular cortices do not target the

spinal cord, which indicates that other brain regions mediate cortical-elicited spinal inhibition. Recent data point to a putative role of the medulla oblongata as a possible relay

of the antinociception produced from those regions. In fact, both telencephalic areas target the DRt and the somatosensory cortex projects, in addition to the RVLM (van Bockstaele and Aston-Jones, 1989; Bernard et al., 1990a; Desbois et al., 1999). The RVLM was shown to inhibit nociceptive spinal neurons (Sidall and Dampney, 1989; Hudson et al., 2000) and the DRt is known to facilitate nociceptive transmission (Almeida et al., 1996, 1999; Lima and Almeida, 2002). The cortical projection to the VLMLat shown in the present study may represent another possible medullary relay in the mediation of the antinociception elicited from the cortex. In a similar manner, the novel pathway from the amygdala to the VLMLat reported in this study may convey the antinociceptive actions from that forebrain region, along with the well-established mediation of the PAG and RMg (Helmstetter et al., 1993a,b; 1998). Ongoing functional experiments will enlighten these questions.

The present data show that the major components of the brainstem endogenous antinociceptive system, namely the PAG, LC, PBN, KF, A<sub>5</sub> noradrenergic cell group, RMg, Sol and VRt (reviewed by Jones, 1992), are VLMLat afferent sources, suggesting that the VLMLat may play an overall function in pain modulation. The specific role in the so-called analgesic mesolimbic loop formed by the CeA, AcB, lateral BST and PAG (Ma and Han, 1991) of VLMLat afferents remains to be ascertained.

Most regions involved in nociceptive control are also important autonomic centers and the application of a noxious stimulus induces cardiovascular changes (Lovick, 1993). The contribution of the VLMLat to the vasodepression produced from the CVLM was demonstrated by showing that glutamate instillation in the VLMLat decreases blood pressure and heart rate while the opposite occurs after local lesion with quinolinic acid (Tavares et al., 1997b). The neuronal circuitry mediating vasodepression from the CVLM has been described (reviewed by Blessing and Li, 1989; Chalmers and Pilowsky, 1991). Afferents from the Sol activate CVLM neurons, which inhibit the vasopressor center located at the RVLM and, therefore, the excitatory effect exerted by the latter at the thoracic intermediolateral cell collum. It is possible that a similar circuit may be involved in the vasodepression produced from the VLMLat since this region was shown here to be targeted by numerous afferents from the Sol and it projects to the RVLM (McKellar and Loewy, 1982; Woulfe et al., 1990). Functional experiments are necessary to ascertain this possibility. A new area recently emerged at the caudal pole of the RVLM, which mediates cerebrovascular vasodilation and is thus known as the medullary cerebrovascular vasodilatory center (Golanov et al., 2000, 2001). Although this region is in the rostromedial continuation of the VLMLat it is likely that they are different regions. Stimulation of the medullary cerebrovascular vasodilatory center induces rises in blood pressure (Golanov et al., 2000) while the opposite effect is produced from the VLMLat (Tavares et al., 1997b).

Beyond the Sol, several brain areas involved in pain control and autonomic functions send afferents to the

VLMLat. They include the IC, CeA, Pa, LH, PAG, LC, PBN, KF, and the A<sub>5</sub> noradrenergic cell group (Jones, 1992; Tavares and Lima, 2002). It is thus possible that the VLMLat can play a role in the integration of both autonomic and nociceptive functions. This putative integrative function of the VLMLat is further supported by the scarcity of afferents received from autonomic regions that do not participate in pain processing. Among such regions, only the BST was shown to send projections to the VLMLat (Ciriello and Jansen, 1993; Roder and Ciriello, 1993; Davis et al., 1994). In line with the proposed nociceptive/cardiovascular integrative role of the VLMLat, studies showed that hypertension-induced hypoalgesia is conveyed by a VLMLat–spinal pathway (Tavares et al., 1997b; Tavares and Lima, 2002).

### Motor-related afferents

The participation of the CVLM in motor functions as been ascribed to the LRt (Clendenin et al., 1974; Alstermark et al., 1981; Ekerot, 1989). In accordance with other tracing studies, our control injections in the LRt showed that it receives massive projections from the main motor-related centers, namely the R, IO and cerebellum. Curiously, many of the somatic motor related centers such as the motor cortices, R, IO, Lat and LRt were here shown to send afferent fibers to the VLMLat. Furthermore, the areas involved in visceral motor control, such as the prelimbic and infralimbic cortices (Hurley-Guis and Neafsey, 1986) were also shown to target the VLMLat. Collectively, these data indicate that, in addition to the LRt, the VLMLat may participate in the motor functions performed by the CVLM. Interestingly, afferents from motor-related regions of the brain target both the VLMLat and the LRt. On the contrary, spinal afferents are topographically segregated in the VLM since fibers from the ventral horn target only the LRt while the VLMLat is targeted exclusively by axons from dorsal horn nociceptive layers (laminae I, IV–V; Tavares et al., 1998; Tavares and Lima 2002). The possible meaning of this dual pattern of projections from motor-related centers remains to be ascertained.

### Afferents from limbic structures

The limbic projections to the VLMLat were here shown to have a wider origin than those targeting other medullary regions, namely the Sol (van der Kooy et al., 1982), LRt (Meyer et al., 1986) and DRt (Almeida et al., 2002). The limbic and infralimbic cortices, the CeA and the BST were all shown to send substantial projections to the VLMLat. Besides the classical involvement of the limbic system in motivational-affective responses to pain (Bernard et al., 1990b; Davis, 1992; Roder and Ciriello, 1993; Davis et al., 1994; Johansen et al., 2001), it was proposed that the system forms an emotional–autonomic network that triggers the autonomic changes observed during emotional challenges, such as fear and anxiety (Touzani et al., 1996; Dun and Williams, 1995; Davis, 1998). Since the medullary areas receiving projections from the limbic system are all involved in cardiovascular control, it is likely that the emotional–autonomic network extends to the medulla oblon-

gata. The inclusion of the VLMLat in that circuit points to its putative involvement in both emotional and cardiovascular reactions to pain.

In summary, the present results indicate that the VLMLat is specially suited to integrate cardiovascular functions, motor activity and affective behavior so that adequate and rapid responses to pain can be produced. The VLMLat may thus play an important role on the protective homeostatic function exerted by the pain system (Melzack and Casey, 1968).

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