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# BRAIN PROJECTIONS FROM THE MEDULLARY DORSAL RETICULAR NUCLEUS: AN ANTEROGRADE AND RETROGRADE TRACING STUDY IN THE RAT

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**Abstract—In the last 15 years a role has been ascribed for the medullary dorsal reticular nucleus as a supraspinal pain modulating area. The medullary dorsal reticular nucleus is reciprocally connected with the spinal dorsal horn, is populated mainly by nociceptive neurons and regulates spinal nociceptive processing. Here we analyze the distribution of brain projections from the medullary dorsal reticular nucleus using the iontophoretic administration of the anterograde tracer biotinylated-dextran amine and the retrograde tracer cholera toxin subunit B.**

**Fibers and terminal boutons labeled from the medullary dorsal reticular nucleus were located predominately in the brainstem, although extending also to the forebrain. In the medulla oblongata, anterograde labeling was observed in the orofacial motor nuclei, inferior olive, caudal ventrolateral medulla, rostral ventromedial medulla, nucleus tractus solitarius and most of the reticular formation. Labeling at the pons-cerebellum level was present in the locus coeruleus, A5 and A7 noradrenergic cell groups, parabrachial and deep cerebellar nuclei, whereas in the mesencephalon it was located in the periaqueductal gray matter, deep mesencephalic, oculomotor and anterior pretectal nuclei, and substantia nigra. In the diencephalon, fibers and terminal boutons were found mainly in the parafascicular, ventromedial, and posterior thalamic nuclei and in the arcuate, lateral, posterior, peri- and paraventricular hypothalamic areas. Telencephalic labeling was consistent but less intense and concentrated in the septal nuclei, globus pallidus and amygdala.**

**The well-known role of the medullary dorsal reticular nucleus in nociception and its pattern of brain projections in rats suggests that the nucleus is possibly implicated in the modulation of: (i) the ascending nociceptive transmission involved in the motivational-affective dimension of pain; (ii) the endogenous supraspinal pain control system centered in the periaqueductal gray matter–rostral ventromedial medulla–spinal cord circuitry; (iii) the motor reactions associated with pain. © 2006 Published by Elsevier Ltd on behalf of IBRO.**

**Key words: supraspinal circuitry, medullary reticular formation, pain pathways, cholera toxin subunit B, biotinylated dextran, brain efferents.**

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*Abbreviations:* (The nomenclature and abbreviations used to designate brain nuclei and fiber tracts are in accordance with those used by Paxinos and Watson (1998) or result from a simplification of it, except for a few exceptions assigned with (*).) ABC, avidin–biotin complex; ac/aca, anterior commissure/anterior part; AH, anterior hypothalamic area; Amy, amygdaloid nuclei; AP, area postrema; APT, anterior pretectal nucleus; Aq, Sylvius aqueduct; Arc, arcuate hypothalamic nuclei; A5/A7, A5/A7 noradrenaline cells; BDA, biotinylated dextran; BLA, basolateral amygdaloid nucleus, anterior part; BST, bed nucleus of the stria terminalis; cc, central canal; Ce, central amygdaloid nuclei; CL, centrolateral thalamic nucleus; CM, central medial thalamic nucleus; CnF, cuneiform nucleus; cp, cerebral peduncle basal part; CPu, caudate putamen; CTb, cholera toxin subunit B; Cu, cuneate nucleus; DK, nucleus of Darkschewitsch; DPAG, dorsal periaqueductal gray; DPGi, dorsal paragigantocellular nucleus; DpMe, deep mesencephalic nucleus; DR, dorsal raphe; DRt, * dorsal reticular nucleus; DTg, dorsal tegmental nucleus; ECu, external cuneate nucleus; En, endopiriform nuclei; Eth, ethmoid thalamic nucleus; f, fornix; F, nucleus of the fields of Forel; fr, fasciculus retroflexus; Gi, gigantocellular reticular nucleus; GiA, gigantocellular reticular nucleus alpha part; GiV, gigantocellular reticular nucleus ventral part; GP, globus pallidus; Gr, gracile nucleus; HDB, nucleus of the horizontal limb of the diagonal band; ic, internal capsule; IC, inferior colliculus; ICj, islands of Calleja; icp, inferior cerebellar peduncle; IG, indusium griseum; IMLF, interstitial nucleus of the medial longitudinal fasciculus; Int, interposed cerebellar nucleus; IO/IOM, inferior olive/medial nucleus; IP, interpeduncular nuclei; IRt, intermediate reticular nucleus; KF, Kölliker-Fuse nucleus; Lat, lateral (dentate) cerebellar nucleus; LC, locus coeruleus; lfp, longitudinal fasciculus of the pons; LGP, lateral globus pallidus; LH, lateral hypothalamus; ll, lateral lemniscus; LL, nuclei of the lateral lemniscus; LM, lateral mammillary nucleus; LPB, lateral parabrachial nuclei; LPGi, lateral paragigantocellular nucleus; LPO, lateral preoptic nuclei; LRt, lateral reticular nucleus; LS/LSI, lateral septal nuclei/intermediate part; LV, lateral ventricle; MCPO, magnocellular preoptic nucleus; Me, medial amygdaloid nuclei; Me5, mesencephalic trigeminal nucleus; MG, medial geniculate nuclei; MGP, medial globus pallidus; ml, medial lemniscus; mlf, medial longitudinal fasciculus; Mo5, motor trigeminal nucleus; MPB, medial parabrachial nucleus; MPO, medial preoptic nuclei; MS, medial septal nucleus; mt, mammillothalamic tract; NTS, * nucleus tractus solitarius; opt, optic tract; ox, optic chiasm; PAG, periaqueductal gray; PB, parabrachial nuclei; PBS, saline phosphate buffer; PBST, 0.1 M saline phosphate buffer containing 0.3% Triton X-100; PC, paracentral thalamic nucleus; PCom, nucleus of the posterior commisure; PCRt, parvicellular reticular nucleus; Pe, periventricular hypothalamic nucleus; PF, parafascicular thalamic nucleus; PGi, paragigantocellular nucleus; PH, posterior hypothalamic area; PHA-L, *Phaseolus vulgaris*-leucoagglutinin; Pn, pontine nuclei; PnC/PnO/PnV, pontine reticular nucleus caudal/oral/ventral part; Po/PoT, posterior thalamic nucleus/triangular part; PO, preoptic nuclei; PPTg, pedunculopontine tegmental nucleus; Pr, prepositus nucleus; PR, prerubral field; Pr5VL, principal sensory trigeminal nucleus, ventrolateral part; PV, paraventricular thalamic nuclei; PVN, * paraventricular hypothalamic nuclei; py, pyramidal tract/decussation; R, red nucleus; Re, reuniens thalamic nucleus; Rh, rhomboid thalamic nucleus; RMg, raphe magnus nucleus; RPa/RObb, raphe pallidus/raphe obscurus; Rt, reticular thalamic nucleus; RVM, rostral ventromedial medulla; SC, superior colliculus; scp, superior cerebellar peduncle; SI, substantia innominata; SN, substantia nigra; SNC, substantia nigra compact part; SNR, substantia nigra reticular part; Sp5, spinal trigeminal nucleus; Sp5C, Sp5 caudal part; Sp5I, spinal trigeminal nucleus interpolar part; SPF, subparafascicular thalamic nucleus; VA, ventral anterior thalamic nucleus; VDB, nucleus of the vertical limb of the diagonal band; Ve, vestibular nuclei; VL, ventrolateral thalamic nucleus; VLH/VMH, ventrolateral/ventromedial hypothalamic nuclei; VLMlat, * lateral portion of the caudal ventrolateral medulla; VLPAG, ventrolateral periaqueductal gray; VM, ventromedial thalamic nucleus; VP, ventral pallidum; VPL/VPM, ventral posterolateral/posteromedial thalamic nucleus; VRt, * ventral reticular nucleus; VTA/R, ventral tegmental area/rostral part; ZID/ZIV, zona incerta dorsal/ventral part; 3, oculomotor nucleus; 7, facial nucleus; 10, dorsal motor nucleus of vagus; 12, hypoglossal nucleus; 3V, 3rd ventricle; 4V, 4th ventricle.

A growing amount of evidence indicates that supraspinal pain control centers involved in the modulation of spinal

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nociceptive transmission can exert both an antinociceptive (inhibitory) and a pronociceptive (facilitating) action upon nociceptive spinal dorsal horn neurons (reviewed by Pertovaara, 2000; Lima and Almeida, 2002; Millan, 2002; Porreca et al., 2002; Gebhart, 2004). In this perspective, the final volume and the characteristics of peripheral nociceptive information reaching thalamic and cortical structures are dependent on the balance between these two opposing influences. Supraspinal brainstem areas like the periaqueductal gray matter (PAG; Bodnar, 2000), rostroventromedial medulla (RVM; Mason, 2001), locus coeruleus (LC; Jones, 1991), lateral portion of the caudal ventrolateral medulla (VLMlat; Tavares and Lima, 2002), dorsal reticular nucleus (DRt; Bouhassira et al., 1992) and nucleus tractus solitarius (NTS; Randich et al., 1988) are well established as being involved in antinociception. However, in recent years, the RVM (Porreca et al., 2002), the NTS (Wiertelak et al., 1997) and the DRt (Almeida et al., 1996; Almeida et al., 1999; Dugast et al., 2003) were shown to exert an additional profound nociceptive facilitating effect upon acute, inflammatory and/or chronic pain.

The DRt has been described in the caudalmost portion of the medullary dorsolateral reticular formation in rats (Valverde, 1962; Newman, 1985; Lima, 1990), monkeys (Villanueva et al., 1990) and humans (Koutcherov et al., 2004). To the best of our knowledge, anatomical, physiological and behavioral studies have solely explored the significant role of the DRt in pain processing and modulation (for reviews see Villanueva et al., 1996; Lima and Almeida, 2002; Monconduit et al., 2002). Anatomical studies have shown reciprocal connections between the DRt and the spinal dorsal horn laminae implicated in nociception (Almeida et al., 1993; Tavares and Lima, 1994; Almeida et al., 1995, 2000; Villanueva et al., 1995; Raboisson et al., 1996; Almeida and Lima, 1997). Electrophysiological studies revealed the presence in the DRt of a population of neurons activated exclusively or mainly by noxious stimuli applied to any part of the body (Villanueva et al., 1988, 1989). Functional studies on the activation of brain areas, measured by the consumption of 2-D-glucose, showed a significant activation of the DRt in inflammatory and chronic pain models (Neto et al., 1999; Porro, 2003). Finally, behavioral studies have implicated the DRt in pain exacerbation through a descending facilitating control of spinal nociceptive transmission in acute and inflammatory pain (Almeida et al., 1996, 1999; Dugast et al., 2003) and the depression of background body sensory activity (diffuse noxious inhibitory control, DNIC; Bouhassira et al., 1992).

The capacity to perform a fine modulatory action upon spinal nociceptive transmission requires a complex network of neuronal connections between brain areas implicated in ascending pain processing and descending pain modulation. Several studies have identified the brain connections of most pain control areas referred above, namely the PAG (Cameron et al., 1995a,b), RVM (Bobillier et al., 1976; Hermann et al., 1997), LC (Jones and Yang, 1985; Luppi et al., 1995), NTS (Menetrey and Basbaum, 1987; Arends et al., 1988; Joseph and Micheal, 1988) and VLMlat (Cobos et al., 2003; Babic et al., 2004). In the case of the DRt, although reciprocal connections with the spinal cord have been studied in detail (see above), only brain afferents to the nucleus have been thoroughly described (Almeida et al., 2002). Concerning efferent connectivity, some brain projections from the DRt have already been described (Bernard et al., 1990) as well as the DRt thalamic projections (Villanueva et al., 1998; Monconduit et al., 2002), both studies based on the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin (PHA-L). However, recent preliminary studies using the anterograde tracer biotinylated dextran (BDA) have clearly shown a DRt brain projecting spectrum much broader then previously thought (Leite-Almeida and Almeida, 2004).

In order to clarify the brain networks that can be used by the DRt to modulate the endogenous pain modulatory circuitry, a detailed analysis of the supraspinal areas receiving axonal projections from DRt was carried out by performing systematic injection of: (i) BDA into different DRt subareas; (ii) BDA in brain areas bordering the DRt; (iii) retrograde tracer cholera toxin subunit B (CTb) into some of the DRt targets demonstrated anterogradely with BDA. Part of the data obtained was previously published in abstract form (Leite-Almeida and Almeida, 2004).

## EXPERIMENTAL PROCEDURES

### Ethical guidelines

Surgical procedures were performed under pentobarbital anesthesia (50 mg/kg, i.p.) on 31 Wistar male rats (Charles River Laboratories, Barcelona, Spain) weighing 280–320 g. Animals were placed in a stereotaxic device (Stoelting, Wood Dale, IL, USA) and a craniotomy was performed. Coordinates for brain injections followed the stereotaxic parameters of (Paxinos and Watson, 1998). The experiments were in accordance with the regulation of local authorities for handling laboratory animals and the European Community Council Directive 86/609/EEC. The number of animals used and their suffering were minimized.

### Anterograde tracing experiments

Fifteen rats received iontophoretic injections (positive direct current of 2.5–3.0 $\mu$A; 5 s on/5 s off, lasting for 10–30 min) of 10% BDA (10,000 MW; Vector Laboratories, Burlingame, USA) in the left DRt through glass micropipettes with 15–20 $\mu$m diameter tips. After completion of the injection period the micropipettes were left *in situ* for 10–15 min before being slowly retracted to avoid tracer reflux along the pipette tract. Two to three weeks later, animals were reanesthetized with 35% chloral hydrate (1 mL/kg body weight) and perfused through the ascending aorta, first with 100 mL of saline phosphate buffer (PBS) 0,1 M, pH 7.2 and then with 1000 mL of 4% paraformaldehyde in PBS. The entire brain was removed, immersed in the same fixative for 4 h and then in 8% sucrose in PBS at 4 °C for 1–2 days. Coronal sections of the entire brain were serially cut on a vibratome at 50 $\mu$m and incubated with 3.3% $H_2O_2$ in order to inhibit endogenous peroxidase. Two in every three successive brain sections were immunoreacted with avidin–biotin complex (ABC, 1:200; Vector Laboratories) for 1 h and then BDA was revealed with 0.0125% diaminobenzidine tetrahydrochloride (DAB; Sigma Immunochemicals, St. Louis, USA) and 0.02% $H_2O_2$ in Tris–HCl buffer 0.05 M, pH 7.6. Half of these sections were counterstained using the formol-Thionin technique (Donovick, 1974) and the remaining were left without any counterstaining. Sections with and without counterstaining were then serially placed in SuperFrost Plus slides (Menzel-Gläser, Braunschweig, Germany), dehydrated and mounted in Entellan (Merck, Darmstadt, Germany).