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Kölliker-Fuse nucleus: the principal source of pontine catecholaminergic cells projecting to the lumbar spinal cord of cat

RICHARD T. STEVENS, CHARLES J. HODGE, Jr.* and APKAR V. APKARIAN

Department of Neurosurgery, Upstate Medical Center, 750 East Adams Street, Syracuse, NY 13210 (U.S.A.)

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Using retrograde transport of the fluorescent dye Evans Blue (EB), in combination with glyoxylic acid histofluorescence, the ponto-spinal catecholaminergic pathways were investigated. The cells which contain catecholamine and project to the lumbar spinal cord of the cat are most densely concentrated in the Kölliker–Fuse nucleus. Locus coeruleus, the subcoeruleus area, and the parabrachial nuclei were found to have relatively few cells that both contain catecholamine and project to the lumbar spinal cord.

Noradrenergic bulbospinal systems have been implicated in a wide variety of spinal segmental functions, including motor reflex control[1,25], modulation of sensory processing[6,12], and vegetative nervous system control[18]. In addition, spinal noradrenaline (NA) has been thought to play a role in opiate and other segmental analgesic mechanisms[10,21,22,27]. Since retrograde and antegrade transport studies have demonstrated a major spinal projection from the area of the noradrenergic nuclei, locus coeruleus (LC) and subcoeruleus (SC), it has been postulated that these nuclei are the primary source of spinal NA[9,16,17,19,23,26]. This postulate has been at least partially confirmed in rat[28]. The inability in cat, however, to block the inhibitory effect of LC stimulation on dorsal horn cell responses by depleting spinal cord NA (Hodge, et al., unpublished observation) suggested that LC might not be the primary source of spinal NA and that some of the LC neurons projecting to the cord are not noradrenergic. This study was undertaken to directly map the locations of cell groups in the dorsolateral pons that both contain catecholamine (CA) and project to the lumbar cord.

Cats were anesthetized with Nembutal and lumbar laminectomies were performed under sterile conditions. The retrogradely transported fluorescent dye Evans Blue (EB) (10% w/v)[4] was injected unilaterally at 8 sites (0.5 μl each) throughout the lumbar gray matter. The animals were allowed to survive 4 days after which they were deeply anesthetized and the brain stems quickly removed and frozen in a dry

* To whom all correspondence should be addressed.

ice/acetone mixture. In contrast to other reports[14,15], the spinal–pontine retrograde transport of EB with a 4-day survival time gave labeling patterns which indicated a sensitivity equivalent to, if not better than, that seen when retrograde transport of horseradish peroxidase, with a 3-day survival time, was used to identify brain stem projections to the lumbar spinal cord (Hodge, et al., unpublished observation). The tissue was sectioned and reacted by a glyoxylic acid technique similar to that of de la Torre and Surgeon[7]. Cryostat sections of the tissue under study, 6–8 μm thick, were collected on clean glass slides and immediately dried with a warm air current for 5 s. The sections were then submerged for 6 s in a 2% glyoxylic acid solution in a phosphate buffer (pH 7.4) and then thoroughly dried with a warm air current. Next, the sides were placed in a 95 °C oven for 6 min, then removed, allowed to cool, and coverslipped with non-fluorescent immersion oil Sections were viewed on a Zeiss Epifluorescence microscope system equipped with a 405/435 nm filter combination for visualizing catecholamines (blue in color) and a 550/590 nm filter combination for visualizing EB (red in color). The data were collected and stored using a PDP-11/34 computer interfaced with x and y potentiometers mounted on the microscope stage[2]. Examination of the injection sites with fluorescence microscopy showed that the EB was located throughout the gray matter on the injected site with some diffusion of the dye to the ipsilateral white matter and contralateral gray matter. The effective unilaterality of the injection, however, was confirmed by examining the red nuclei.

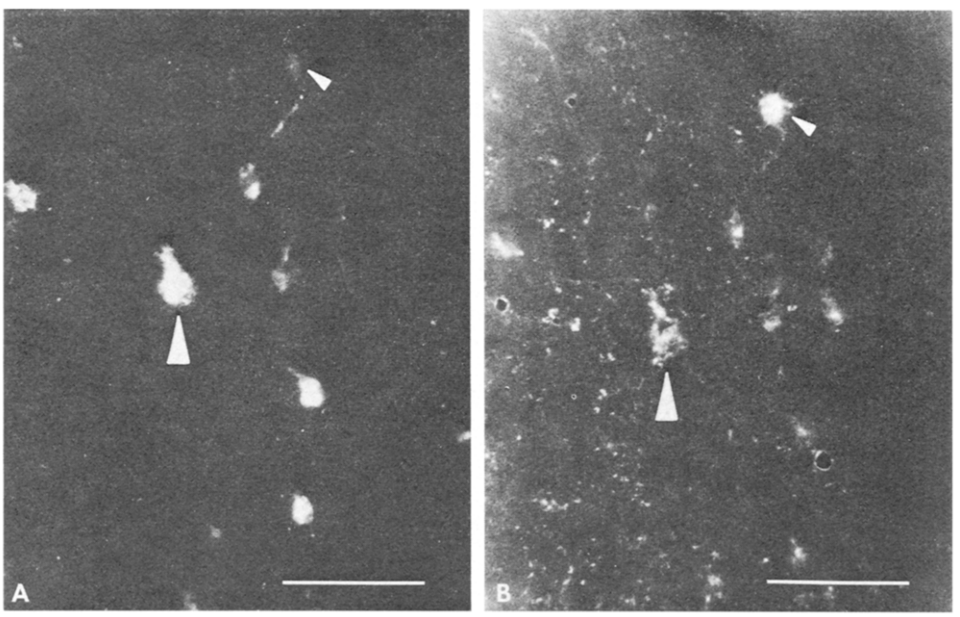

Fig. 1. Two photographs of the same field in the area of the Kölliker–Fuse nucleus using different filter combinations., A: Evans Blue-labeled cells viewed with a 550/590 nm filter combination. B: catecholamine-containing cells demonstrated by a 405/435 nm filter combination. Large arrow indicates a CA cell which projects to the lumbar spinal cord, while the small arrow indicates a CA cell which is not labeled with Evans Blue. Calibration bar is 100 μm.

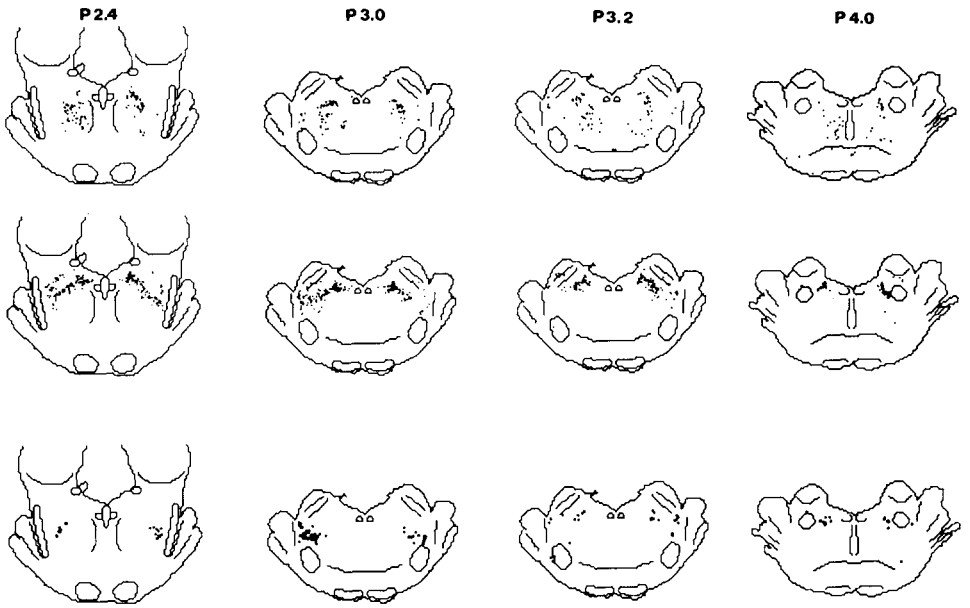

Fig. 2. Outlines of 4 pontine levels (P2.4 to P4.0) viewed from below on which are plotted the locations of cells projecting to the lumbar spinal cord but not containing catecholamine (top row), the locations of cells containing catecholamine but not projecting to the lumbar spinal cord (middle row), and the locations of cells which both contain catecholamine and project to the lumbar spinal cord (bottom row). The data for each pontine level was taken from a single 6–8 μm section. Each mark represents one cell. The EB injections were on the left side of the animal's lumbar enlargement.

Results were accepted from cats where only the red nucleus contralateral to the injection site contained retrogradely labeled cells.

Fig. 1 shows a single field in the area of the Kölliker–Fuse nucleus viewed with each of the two filter combinations. On the left (A) are several cells which project to the lumbar cord, and on the right (B) it can be seen that most of these are catecholaminergic. Fig. 2 shows the locations of cells, at 4 pontine levels, which contain retrogradely transported EB or which contain CA, or which contain both CA and retrogradely transported EB. Throughout the dorsolateral pons there is a dense concentration of CA-containing cells extending as a band from LC, dorsally, to the more ventral and lateral groups, SC, and the medial parabrachial nucleus (PBM) as previously described[20]. The most ventral group of CA cells, located at the ventral margin of the brachium conjunctivum, is in the region of the Kölliker–Fuse (KF) nucleus[3]. There are relatively few CA cells in the lateral parabrachial nucleus (PBL). At the more caudal level (P 4.0) CA cells are mostly found in LC. The vast majority of CA cells in LC, SC and PBM do not project to the lumbar cord. There are large numbers of non-noradrenergic cells of the dorsolateral pons which project to the cord. These cells are located just medial to the CA cell groups, though there is some overlap in the border zone between these groups (see also Fig. 3). These cells projecting to the lumbar spinal cord are found bilaterally, though there is some apparent ipsilateral predominance. Catecholaminergic cells projecting to the lumbar cord are found primarily in the KF nucleus, located just dorsal to the ventral nucleus of the lateral

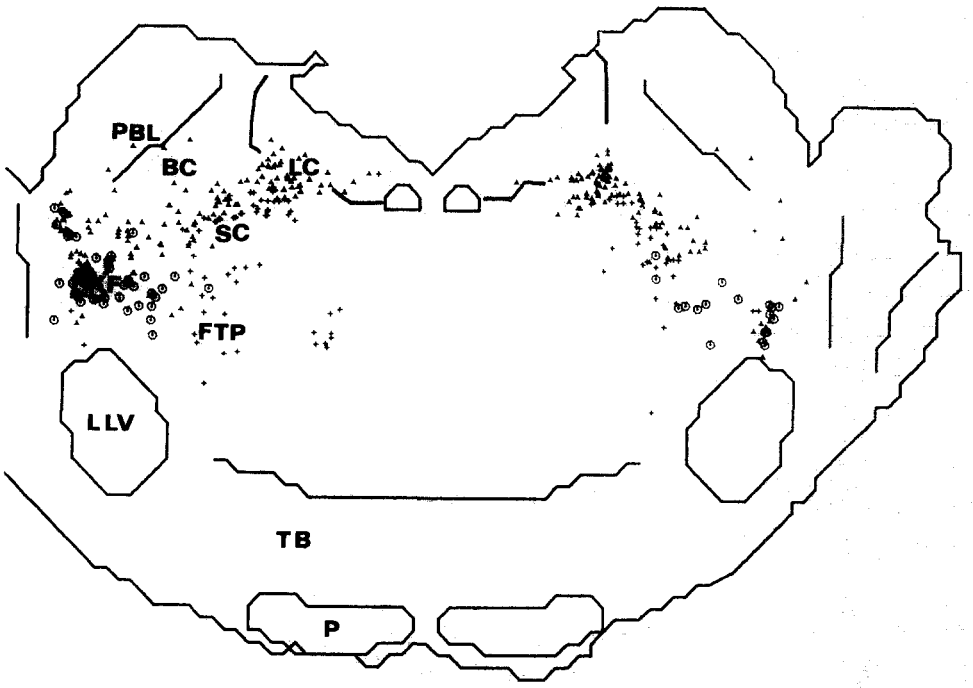

Fig. 3. An outline of the pons (P3.0) viewed from below on which are plotted the locations of cells containing retrogradely transported Evans Blue but not catecholamine (+), the locations of cells containing catecholamine but not Evans Blue (▲), and the locations of cells containing catecholamine and retrogradely transported Evans Blue (⊙). The data for this figure are from a single 6-8 μm section. Each point represents one cell. The Evans Blue injections were made in the left side of the cat's lumbar enlargement. BC, brachium conjunctivum; FTP, paralemniscal tegmental field; KF, Kölliker–Fuse nucleus; LC, locus coeruleus; LLV, nucleus of the lateral lemniscus; P, pyramid; PBL, lateral parabrachial nucleus; SC, nucleus subcoeruleus; TB, trapezoid body.

lemniscus (LLV). In the more caudal pons there are cells in LC, SC and PBM which contain CA and project to the lumbar spinal cord, but the number and density of these is much less than that found in KF. The glyoxylic acid technique seemed as sensitive as the Falck–Hillarp method for identifying CA-containing cells, since the density and distribution we found of these cells is similar to that described by others using the Falck–Hillarp method[5,13,22]. The cells, which contain CA and project to the lumbar spinal cord, seen in KF as well as LC, SC and PBM, are located bilaterally but with an ipsilateral predominance.

These results indicate that, in cat, the majority of pontine catecholaminergic cells projecting to the lumbar cord are located in KF and not in LC or SC. The studies purporting to show that the major noradrenergic spinal projection is from LC are misleading because of the interspersion and proximity of non-projecting CA-containing cells and projecting non-CA cells. The assumption, then, that stimulation or destruction of the immediate LC area affects spinal mechanisms by directly altering only descending spinal noradrenergic innervation[11,24,25] is not substantiated. The studies of Hammond and Proudfit[8] have addressed this issue. These discrepancies may not apply in other species where direct histochemical studies havre shown noradren-

ergic coeruleospinal projections[28]. Cells located in the cat Kölliker–Fuse nucleus have previously been shown to contain CA[5,13] and have been demonstrated to project to the spinal cord[9,26]. While the entire dorsolateral pontine CA system has been regarded as a homogeneous group of cells[13], the presence of differential projections from the KF nucleus to the cord suggests that this area can be subdivided into functional groups.

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