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Spinal Projections of Brainstem Respiratory Related Neurons in the Cat as Revealed by Retrograde Fluorescent Markers*

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By means of retrograde axonal transport of fluorescent tracers, connections between brainstem respiratory related regions and the spinal cord has been studied in the cat. Neurons at the pneumotaxic center project bilaterally (90 % ipsi-, 10 % contra-) to cervical and lumbar spinal cord and ipsilaterally to thoracic levels. The ventrolateral nucleus of the tractus solitarius project mainly contralaterally (85 %) to cervical levels and only contralaterally to thoracic levels; no efferent projections were found to lumbar levels. The ventral respiratory group showed a great number of neurons projecting to the spinal cord especially from the nucleus retroambiguus. Both nuclei, ambiguus and retroambiguus, project mainly contralaterally (70 %) to the spinal cord. The Bötzinger complex showed rather scarce bilateral projections to cervical and only ipsilateral projections to lower cervical, thoracic and lumbar levels.

Key words: Respiratory related nuclei, Brainstem, Spinal projections, Fluorescent markers. Cat.

Although there are numerous neuroanatomical (10, 13, 14, 15) and electrophysiological (3-6, 9, 12, 16) reports about the efferent projections of pontomedullary centers to the spinal cord, the present picture of these projections is rather fragmentary. For example, quantitative data concerning the relative contribution of brainstem respiratory nuclei to the spinal cord are still needed. Using retrograde fluorescent markers it is possible to make injections restricted to small areas of the spinal cord but still maintaining a good ratio of labeled neurons in the brainstem (11). The aim of the present report has been to study the spinal projections of the pneumotaxic center (nucleus parabrachialis medialis and Kölliker-Fuse nucleus, NPBM-KF), of the dorsal respiratory group (ventrolateral nucleus of the tractus solitarius, vlNTS) and of the ventral respi-

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ratory group (nucleus retroambiguus, NRA; nucleus ambiguus, NA and Bötzinger complex, Bc). Spinal projections of respiratory pontomedullary centers were studied using fluorescent retrograde markers injected into the spinal cord.

Materials and Methods

Experiments were carried out on 12 adult cats weighing 1.7 to 5 kg. Under general anesthesia (Ketamine, 35 mg/kg, i.m.) laminectomies were made at four different levels of the spinal cord (C1-C3, C4-C6, T6-T8 and L1-L3). 0.5-1 µl of 3 % Fast Blue (FB) (11) or 2 % diamidino yellow dihydrochloride (DY) were injected into the lateral or ventral reticulo spinal tract (1 or vRST) and/or the ventral horn with the help of a Hamilton microsyringe (table I). After appropriate survival time (3 days per cm of injection site location from the obex) animals were deeply reanesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and perfused intracardially with saline followed by 10 % formalin (pH 7.2). The brainstems and spinal cords were removed and cut serially in a freezing microtome at 50 µm sections. Sections were mounted on gelatin coated slides, air dried and coverslipped. Every third section was counterstained with cresyl violet for reference purposes (1). Sections between spinal level C1 and the pons were studied with a Leizt Ploemopack fluorescence microscope with a filter system A (360 nm wavelength) for the presence of somata fluorescing in blue and nuclei fluorescing in yellow (8, 11).

Results

The distribution of labeled neurons following the injection of FB or DY into different levels of the spinal cord was mapped onto outline coronal drawings of the brain-

Tabla I. *Labeled neurons found at different brainstem nuclei after the injection of retrograde axonal markers into different levels of cat's spinal cord.*

Injection site	Evidence	NPBM-KF		vlNTS		NRA		NA		Bc		Source
		i	c	i	c	i	c	i	c	i	c	
(C_1-C_3)	FB	+++	(+)	(+)	++	++	+++	(+)	++	(+)	(+)	PR
	DY	++++	(+)	(+)	+	(+)	++++	+	+	(+)	(+)	PR
	HRP	+++	(+)	(+)	+	—	—	(+)	(+)	—	—	(10, 14, 15)
	Elec.	—	—	(+)	++	(+)	++	—	++	(+)	+	(2, 3, 4, 6, 12)
(C_4-C_6)	FB	+++	(+)	(+)	++	++	++++	(+)	(+)	(+)	0	PR
	HRP	++	+	+	++	—	—	(+)	(+)	—	—	(15, 13)
	Elec.	—	—	(+)	++	(+)	++	—	—	—	—	(4, 5)
(T_7-T_8)	FB	++	0	0	(+)	(+)	+++	0	(+)	(+)	0	PR
	HRP	++	(+)	+	++	—	—	(+)	(+)	—	—	(10, 15)
	Elec.	—	—	0	++	0	++	—	+	—	—	(7, 9)
(L_1-L_3)	FB	+++	(+)	0	0	0	(+)	0	(+)	(+)	0	PR

Abbreviations: NPBM-KF, nucleus parabrachialis medialis and Kölliker-Fuse nucleus; vlNTS, ventrolateral nucleus of tractus solitarius; NRA, nucleus retroambiguus; NA, nucleus ambiguus; Bc, Bötzinger complex; IRST, lateral reticulo spinal tract; FB, fast blue; DY, diamidino yellow; HRP, horseradish peroxidase; Elec., electrophysiological methods; PR, present reults; i, ipsilateral and c, contralateral to the injection sites. (+), 1-25; +, 26-50; ++, 51-75; +++, 76-100; ++++, 101-125 neurons respectively.

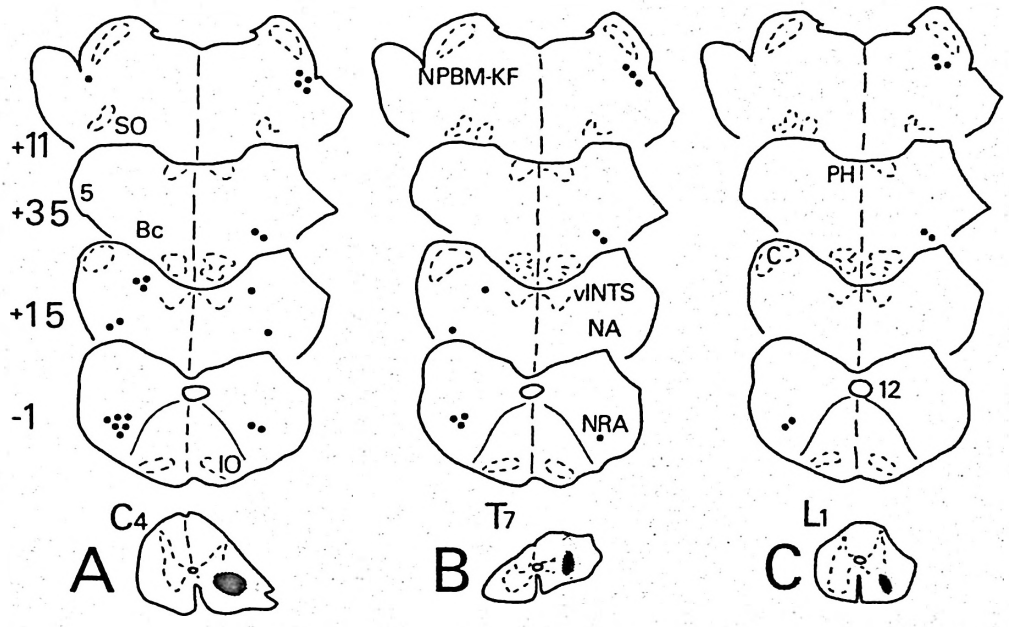

Fig. 1. *Distribution of retrogradely labeled neurons (each dot represents 2 labeled neurons) at several brainstem coronal sections (each section was 180 μm thick) after unilateral injection of Fast Blue at three different levels of cat's spinal cord.*

A, C4; B, T7 and C, L1. Coordinates: distance in mm respect to the obex (+ rostral, − caudal). 5, spinal trigeminal tract; 12, hypoglossal nerve; Bc, Bötzinger complex; C, nucleus cuneatus; IO, inferior olive; NA, nucleus ambiguus; NPBM-KF, nucleus parabrachialis medialis and Kölliker-Fuse nucleus; vlNTS, ventrolateral nucleus of tractus solitarius; PH, prepositus hypoglossi nucleus; SO, superior olive. Dark areas of the injection site show the deposit of fluorescent marker and shaded zons represent the spreading of the tracer.

stem (fig. 1). Only labeled neurons located in the above mentioned brainstem respiratory related nuclei were considered in the present report. The relative density of labeled neurons found in the four different experimental groups are shown in table I. The number of labeled neurons was smaller after thoracic and lumbar than after cervical injections, but their distribution was roughly the same (fig. 1). Labeled neurons showed multipolar, fusiform and pyramidal somatas (fig. 2) without any special distribution in studied nuclei. DY seems to label more neurons than FB.

Although in the NPBM-KF, labeled neurons were found mainly ipsilaterally (90 %) to the injection site, a weak (10 %) contralateral projection was also observed, but restricted to cervical and lumbar levels. More neurons were found at the KF nucleus after cervical and thoracic injections than in the NPBM; after lumbar injections, more neurons were found at the NPBM than in the KF. In the vlNTS, labeled neurons were found mainly contralateral (85 %) to the injection site. Contralateral, but not ipsilateral, vlNTS neurons were labeled after FB injections in the thoracic spinal cord. No labeled neurons were found at the vlNTS after lumbar injection. Labeled neurons were found through the whole extent of the ventral respiratory group following FB or DY injections in the spinal cord. NRA showed the greatest number of labeled cells, especially after the injection at the level of the phrenic nucleus (Large FB injections in this nucleus, including both 1 and vRST). NRA labeled

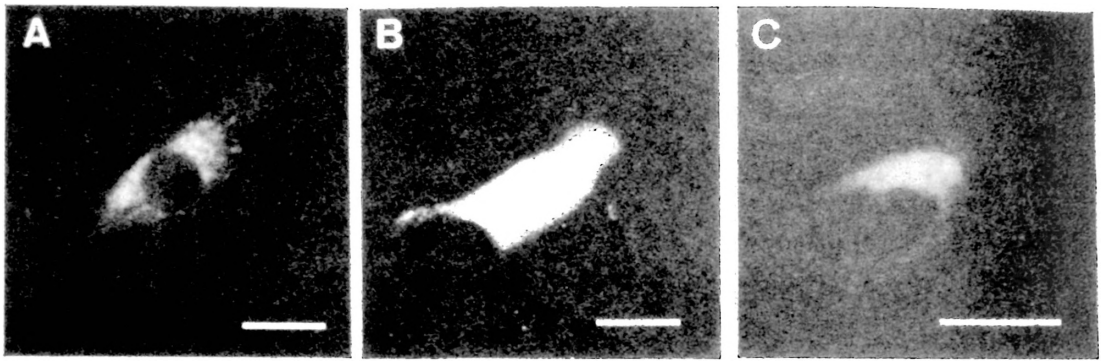

Fig. 2. *Photomicrographs of labeled neurons following Fast Blue (FB) injections at the cat's spinal cord.* A, B and C, examples of fusiform, multipolar and pyramidal neurons. Bar 25 μm.

cells (70 %) were located contralaterally to the injection site. NA neurons were labeled preferentially (70 %) after FB injections in the contralateral spinal cord, but a few ipsilateral cells (30 %) were also labeled. Any homolateral labeled neurons were found after lumbar injections. Bc neurons were bilaterally labeled after injection in C1-C3, but the label was exclusively ipsilateral at the lower injected sites.

Discussion

Table I includes a summary of recent electrophysiological and morphological descriptions of ponto-medullary respiratory nuclei projections to the spinal cord. For the sake of comparison with present results an approximate evaluation of reported projection densities has been made. Obviously for technical reasons electro-

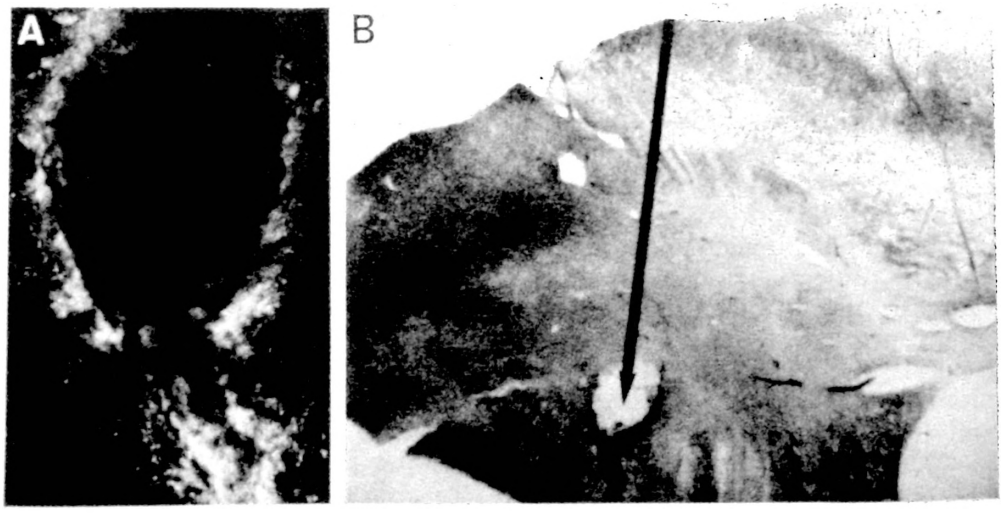

Fig. 3. *Photomicrographs of spinal cord section (C4) showing the injection site of Fast Blue retrograde fluorescent neuronal marker (FB).*

A, FB injection (0.5 μl) under fluorescing microscope (360 nm wave length) (× 60). B, the same injection site as A under light microscope, showing the trajectory of the microsyringe (black arrow) (× 15).

physiological reports included in table I were rather imprecise about localization of recorded neurons, especially in relation to the ventral respiratory group.

Present findings show that neurons in the NPBM-KF give rise to long descending fibers which travel, mainly but not exclusively, through the ipsilateral half of the spinal cord. The contralateral pathway seems to be more restricted to cervical levels. Similar results have been reported (15), although other studies using different neuroanatomical methods failed to demonstrate contralateral connections between NPBM-KF and the spinal cord (10, 14).

Based on electrophysiological experiments a contralateral projection has been described for the vlNTS to respiratory motor nuclei in the spinal cord (3, 7, 16). However, previous morphological studies (10, 13, 15) and present results have demonstrated the presence of an important ipsilateral projection to the same sites. Any efferent projections have been shown to the lumbar site.

Spinal projections of the different nuclei of the VRG have been studied with both electrophysiological and morphological techniques (4-6, 10, 12, 13, 15). In electrophysiological experiments, the strong NRA projection to the spinal cord probably masked the more scarce NA projection to the same spinal levels. The presence of a NA projection to the spinal cord was described previously using electrophysiological techniques (6), although it was not observed by others (12). On the other hand, these projections were supposed to be exclusively contralateral, except for the Bc (5). Present experiments have demonstrated the presence of ipsilateral projections both for the NRA and NA to the spinal cord, a fact not well documented in previous morphological reports using HRP as a retrograde tracer (10, 13, 15). Although not previously described using morphological techniques, according to the present report a weak bilateral projection seems to exist from the Bc to the spinal cord; this projection becomes ipsilateral at lower spinal levels and was described previously with electrophysiological techniques (4).

Resumen

Se estudia mediante la técnica de marcadores fluorescentes retrógrados, las conexiones entre las regiones del tronco del encéfalo relacionadas con la respiración y la médula espinal del gato. Las neuronas del centro neumotáxico proyectan bilateralmente (90 % homo- 10 % contra-) a la médula espinal cervical y lumbar, y homolateralmente a los niveles torácicos. El núcleo ventrolateral del tracto solitario proyecta, principalmente, contralateralmente (85 %) a los niveles medulares cervicales y sólo contralateralmente a los niveles torácicos. No se encuentran proyecciones eferentes hacia los niveles lumbares de la médula espinal. El grupo respiratorio ventral muestra una gran cantidad de neuronas que proyectan hacia la médula espinal, especialmente a partir del núcleo retroambiguo. Los núcleos ambiguo y retroambiguo proyectan principalmente (70 %) a la médula espinal cervical contralateral. El complejo de Bötzinger presenta proyecciones bilaterales escasas hacia los niveles cervicales superiores y solamente homolaterales hacia los niveles cervicales inferiores, torácicos y lumbares.

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