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Brainstem neurons with projecting axons to both phrenic and abdominal motor nuclei: a double fluorescent labeling study in the cat

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Abstract

The distribution of retrogradely doubly labeled brainstem neurons were analyzed in the cat after injection of two different fluorescent markers into the phrenic and abdominal motor nuclei. Diamidino Yellow (DY) was first injected either ipsilaterally or bilaterally into the ventral horn of lumbar spinal cord, and then Fast Blue (FB) into the right ventral horn of cervical spinal cord. Doubly labeled neurons were mainly found in the caudal ventrolateral medulla (retroambiguus region), in the dorsomedial and dorsolateral regions of the nucleus of the tractus solitarius (NTS) and in the raphe nuclei. In addition, doubly labeled neurons were found in the parabrachial and Kölliker-Fuse nuclei. Our results give anatomical evidence that pontine and medullary neurons are the source of a common pathway to both phrenic and abdominal motor nuclei. These neurons might be involved in strain efforts for expulsion such as vomiting or defecation.

Key words: Brainstem; Cat; Fluorescent marker; Double labeling; Phrenic motor nucleus; Abdominal motor nucleus

In mammals, large increases in intra-abdominal pressure serving the realization of different strains of expulsion such as vomiting are generated by the main respiratory muscles. During vomiting the diaphragm and abdominal muscles (i.e., internal and external oblique, and rectus abdominis) the contraction of which alternate during ventilation, co-contract in a series of bursts of activity (retching phase) that culminates in expulsion [17,19,25]. Several electrophysiological studies [12,13,17,27] have revealed that intercostal muscles are also activated during vomiting. However, their precise timing of activation is still controversial [11]. Patterns of activity of the portion of the diaphragm that surrounds the oesophagus are also changed during vomiting [21,28,32] as well as activity of upper airway musculature [10,11]. The location of the neuronal network which generates and co-ordinates the motor act for expulsion behaviors is still under investigation. Studies involving lesions or electrical stimulations of brainstem structures have demonstrated that vomiting is induced by a neuronal circuitry entirely located within the medulla [20], while defecation can be abolished by pons lesioning [7]. However, the cell groups involved in these reflexes developing strains for expulsion remain unrecognized.

Recently, it has been shown in the decerebrate cat that during 'fictive' vomiting in paralyzed, decerebrate preparation, the medullary inspiratory premotor neurons in both the dorsal (DRG) and ventral respiratory group (VRG), which normally drive the phrenic motoneurons during ventilation, are strongly inhibited [2,22]. These results suggest that phrenic motoneurons receive during vomiting an excitatory input arising from neurons located in unidentified areas of the brainstem.

In order to localize, in the pons and the medulla, those neurons which might be involved in the realization of the co-activation of phrenic and abdominal motoneurons during strains for expulsion, we analyzed the distribution of brainstem neurons that could exhibit projecting axons to both phrenic and abdominal motor nuclei.

Injections of 50–100 nl of two different fluorescent

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markers (FB, 3% solution and DY, 3% suspension in distilled water) were performed in the spinal cord of 24 male cats weighing 2.5–3.5 Kg. The animals were anesthetized with Saffan (1.5 ml/kg i.m., Pitman-Moore) and halothane (Fluothane), and were subjected to surgery under aseptic conditions. Diamidino Yellow (DY) was first injected in the right (Group 1) or in right and left (Group 2) ventral horns of the lumbar (L1–L3 level) spinal cord (0.5–0.9 mm to the midline and 2.3–2.9 mm under the dorsal surface) by means of a glass micropipette connected to a pressure injector device or to a Hamilton syringe moved with a micromanipulator. Accordingly to the longitudinal extension of the phrenic [5,9] and abdominal motoneurons [18], in Group 2 experiments, several injection sites were done in both cervical and lumbar spinal cord to enhance the probability of retrograde labeling of brainstem neurons. After occlusion of the surgical approach, the animals were unanesthetized and allowed to recover. Total recovery occurred in 24 h and the animals were got back to their breeding room. Three weeks later, the animals of Groups 1 and 2 were re-anesthetized and Fast Blue (FB) solution was injected in the right ventral horn of the cervical (C4–C6 level) spinal cord (0.9–1.5 mm to the midline and 3.5–4.0 mm under the dorsal surface). Correct placements of the micopipettes were attested in some animals by prior recording with a tungsten microelectrode (10 megohms) of either expiratory gross activity (lumbar spinal cord injection) or inspiratory activity (cervical spinal cord injection); in two animals we also used antidromic field potentials obtained in the lumbar spinal cord after stimulation of one branch of the iliogastric nerve (L1). The micropipettes containing the fluorescent markers were then positioned with the same coordinates similar to those used to get either respiratory or field potentials

After recovery from this second surgery and anesthesia, animals were allowed to survive three additional weeks, and then sacrificed under lethal dose of barbiturate. The animals were perfused through the aorta with saline, buffered fixative (pH 7.4) and then 10% sucrose solutions. The cervical and lumbar spinal cord containing site of injections, and brainstem were removed, kept overnight in 30% sucrose solution and cut transversely at 100 μm and 50 μm, respectively. All sections were mounted on gelatine-coated slides, air-dried and covered with Depex. Anatomical landmarks of cerebral structures were determined by counterstaining one of six sections using Berman's atlas [1]. The other sections were examined using a microscope equipped with epifluorescence (excitation wavelength: 365 nm). Correct placement of the injection sites within cervical and lumbar spinal cord were verified and considered as positive if they were restricted to the ventral horn. Extension of the injection site was limited to 0.5–1.0 mm in the dorsoventral direction, and 0.3–0.5 mm in the other directions. The presence of FB or DY single retrogradely labeled neurons, and FB and DY double retrogradely labeled neurons was determined within the brainstem, and reconstruction of their site of location was done using a camera lucida attachment (Fig. 1).

Table 1
Average number (n, total number of cells/number of animals) of labeled cells in brainstem nuclei obtained for three group 1 animals and two group 2 animals

	Group 1						Group 2					
	FB		DY		FB-DY		FB		DY		FB-DY	
	n	range	n	range	n	range	n	range	n	range	n	range
Retroambiguus nucleus region	43.3	(13–79)	55.3	(10–92)	0.3	(0–1)	200.5	(42–379)	219.0	(180–273)	3.0	(1–5)
Ambiguus nucleus region	32.3	(2–58)	46.0	(0–111)	–	–	260.0	(63–457)	4.5	(2–9)		
Retrofacial nucleus	8.3	(4.16)	26.3	(15–42)	–		17.2	(0–35)	9.5	(0–19)		
Ventrolateral solitary tract	5.3	(6–24)	22.6	(1–54)	–		164.5	(41–288)	29.0	(26–32)		
Dorsolateral solitary tract	0.6	(0–2)	3.3	(0–9)	–	–	39.5	(17–62)	78.0	(59–97)	3.5	(0–7)
Dorsomedial solitary tract	7.3	(1–17)	14.3	(8–26)	0.6	(0–1)	20.5	(9–32)	30.0	(11–49)	4.5	(1–8)
Inferior central n. (raphe magnus)	3.6	(0–6)	4.3	(1–12)	–	–	66.5	(36–97)	86.0	(80–92)	7.0	(3–11)
Postpyramidal raphe nucleus	1.0	(0–3)	7.6	(0–22)	–	–	212.0	(147–277)	196.0	(195–197)	12.5	(12–33)
Paramedian reticular nucleus	21.0	(0–63)	78.0	(0–235)	–	–	20.5	(6–35)	29.5	(4–55)		
Lateral tegmental field	6.3	(0–19)	11.6	(0–35)	–		6.5	(5–8)	17.5	(11–24)		
Magnocellular tegmental field	18.3	(8–34)	20.6	(2–59)	–		15.0	(7–23)	42.5	(35–50)		
Gigantocellular tegmental field	2.6	(0–6)	6.0	(0–17)	–		13.5	(6–21)	6.0	(4–8)		
Lateral reticular nucleus	10.0	(0–30)	6.0	(0–14)	0.3	(0–1)	43.0	(6–80)	41.5	(6–77)	3.0	(0–6)
Spinal trigeminal nucleus	1.3	(0–4)	–	–	–		10.0	(2–18)	2.5	(2–3)		
Inferior vestibular nucleus	8.3	(0–23)	8.0	(0–22)	0.3	(0–1)	3.5	(0–7)	5.5	(0–11)	1.0	(0–2)
Medial parabrachial nucleus	23.0	(12–30)	19.6	(18–21)	3.0	(1–5)	135.0	(16–254)	288.5	(48–529)	21.0	(2–40)
Lateral parabrachial nucleus	11.0	(3–26)	11.6	(2–24)	2.0	(0–5)	69.5	(46–93)	112.5	(104–121)	18.0	(3–33)
Kölliker–Fuse nucleus	49.3	(15–69)	36.6	(17–54)	1.8	(1–6)	102.5	(83–122)	219.5	(171–268)	20.5	(11–30)
Paralemniscal tegmental field	4.3	(0–13)	0.6	(0–2)		–	20.0	(2–38)	5.5	(5–6)		
Caeruleus nucleus	6.3	(0–19)	9.3	(0–28)	–	–	4.0	(1–7)	16.0	(8–24)		

Range columns give minimum and maximum number of labeled cells in the different animals studied in each group.

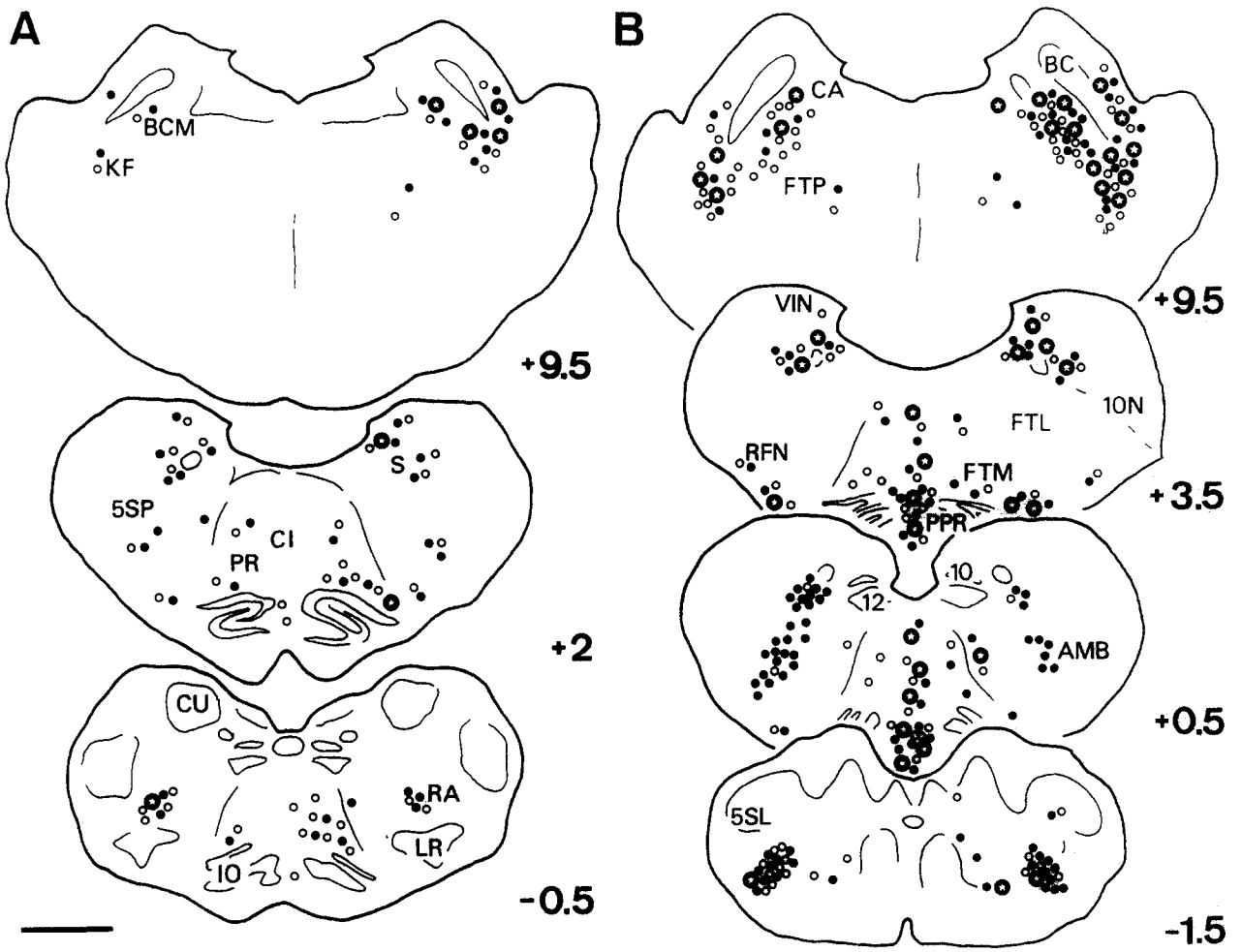

Fig. 1. Exemples of distribution of single FB (black circles), DY (open circles) and double (stars) retrogradely labeled neurons in diagrams representing coronal sections of the brainstem of two cats. A: group 1 cat, after right cervical and lumbar injection of FB and DY, respectively. B: group 2 cat, after right cervical injection of FB and bilateral lumbar injection of DY. Each circle or star represents a maximum of 20 single labeled neurons, or 3 double labeled neurons, respectively. Numbers indicate in mm the rostrocaudal position of the sections, zero being the obex. 5SL, laminar spinal trigeminal nucleus; 5SP, alaminar spinal trigeminal nucleus; 10N, vagus nerve; 10, motor dorsal nucleus of the vagus; 12, hypoglosal nucleus; AMB, nucleus ambiguus; BC, brachium conjunctivum; BCM marginalis nucleus of the brachium conjunctivum; CA, nucleus caeruleus; CI, inferior central nucleus; CU, cuneate nucleus; FTL, lateral tegmental field; FTM, magnocelular tegmental field; FTP, paralemniscal tegmental field; IO, inferior olive; KF, Kölliker-Fuse nucleus; LR, lateral reticular nucleus; PPR, postpyramidal nucleus of the raphe; PR, paramedian reticular nucleus; RFN, retrofacialis nucleus; RA, retroambiguus nucleus; S, solitary tract; VIN, inferior vestibular nucleus. Bar = 2 mm.

Table 1 summarize average numbers of FB or DY single, and FB and DY double retrogradely labeled neurons observed in the five experiments in which correct placement of both markers in the spinal cord of the same animal were considered as positive, and in which double labelings were detected. However, in those experiments in which only one injection site was positive, only single labelings were observed in regions corresponding to the solitary complex and/or, ambiguus and retroambiguus nuclei, thus justifying the validity of the method.

When the two fluorescent markers were applied to the same side of the lumbar and cervical cord (Group 1 experiments), single labeled medullary neurons were located bilaterally in the ventral respiratory group (VRG), i.e. region of the retroambiguus, ambiguus and retrofacial nuclei, in different subnuclei of the nucleus of the tractus solitarius (NTS), and in the magnocellular tegmental field and paramedial reticular nucleus. In the pons, single labeled neurons were mainly located ipsilaterally in the lateral and medial nucleus of the brachium conjunctivum (parabrachial) and Kölliker-Fuse nucleus. A few neurons were also single labeled in the postpyramidal raphe nucleus, inferior central nucleus (raphe magnus), inferior vestibular nucleus, lateral tegmental field, gigantocellular tegmental field, and lateral reticular nucleus. A few FB and DY doubly labeled neurons were also found in these Group 1 experiments. They were located in the contralateral caudal VRG (retroambiguus region), dorsomedial part of the ipsilateral NTS and in the lateral reticular formation (Fig. 1A).

When FB was injected in one side of cervical spinal cord and DY in both sides of the lumbar spinal cord (Group 2 experiments), single medullary labeled neurons were found in same regions as described for Group 1 experiments. However, larger number of neurons were labeled due the multiple spinal injection sites, and numerous bilateral DY single labeled neurons were also observed. Doubly labeled neurons were larger in number compared to the Group 1 experiments. They were similarly located in medullary regions, i.e. retroambiguus, reticular lateral nucleus, dorsomedial NTS and inferior vestibular regions, and in pontine regions, i.e. lateral and medial nucleus of the brachium conjunctivum (parabrachial nuclei) and Kölliker-Fuse nucleus. In addition, number of doubly labeled neurons were found in the raphe magnus, postpyramidal raphe nucleus and dorsolateral portion of the NTS (Fig. 1B).

The results of the present study give anatomical evidence that pontine and medullary neurons are the source of a common descending pathway to both the phrenic and abdominal motor nuclei. Some of the bulbospinal neurons doubly labeled and projecting axons to cervical and lumbar spinal cord might be involved in strain efforts for expulsion developed in various behaviors such as vomiting, coughing, micturition, parturition or defecation.

Doubly labeled neurons were found in medullary regions from which it has been shown by electrical microstimulation that vomiting could be induced in cat [3,26] or in dog [14]. These regions include the nucleus of the tractus solitarius which is considered as a key structure in the induction and control of vomiting [4], and the reticular formation adjacent to it. In our neuroanatomical experiments we also observed number of double retrogradely labeled neurons in postpyramidal (raphe pallidus) and central inferior (raphe magnus and obscurus) raphe nuclei. This confirms previous electrophysiological [15] and immunohistochemical [29] studies describing serotonergic descending inputs on phrenic motoneurons arising from raphe neurons. The connection originated from raphe magnus neurons might be used to prevent elicitation of pain during sudden and potent activation of the diaphragm developed for instance during vomiting or coughing, while that from raphe pallidus neurons might be involved in cardiovascular adjustements concomitant to the accomplishment of these motor activities. These hypothesis are supported by recent investigations describing an intense positive c-Fos-like immunoreactivity in the caudal raphe nuclei (i.e. magnus and obscurus) in response to repetitive episodes of vomiting [23] and coughing [8], and in the nucleus raphe pallidus during moderate hypercapnia [16].

We also found number of doubly labeled neurons in pontine structures such as the parabrachial and Kölliker-Fuse nuclei. Experiments in which these nuclei were destroyed or stimulated indicated that these structures are involved in the control of straining for defecation, a reflex which also induces co-contraction of inspiratory and expiratory muscles [6,7].

Our study also showed single retrogradely labeled neurons whose location fits well with previous results from other groups. Indeed, our results are consistent with the medullary bulbospinal projections of neurons of the rostral VRG and DRG to the phrenic nucleus [30,31] and from the caudal VRG to the abdominal motoneurons [24,30]. Interestingly, no doubly labeled neuron was found in the rostral VRG or in the ventrolateral part of the NTS. This is consistent with the observation that inspiratory bulbospinal neurons do not participate to vomiting since they are strongly inhibited during co-activation of phrenic and abdominal motoneurons [2,22]

Further investigations using electrophysiological recording techniques are now necessary to establish the involvement of those doubly labeled brainstem neurons in the control of strain behaviors for expulsion.

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