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Electrophysiological study of dorsal respiratory neurons in the medulla oblongata of the rat

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Abstract

There has been controversy whether the dorsal respiratory group (DRG), identified in the cat and several other species as a concentration of mainly inspiratory neurons located in the ventrolateral subnucleus of the solitary tract, also exists in the rat. The aim of this study was to re-examine this question by systematically exploring this region with extracellular microelectrodes, in anesthetized and artificially ventilated rats. One-hundred and forty-two units were recorded which fired in phase with central respiratory cycles (determined by recording from the phrenic nerve) and/or lung inflations. One-hundred and nineteen recordings were thought to be from neuronal cell bodies (confirmed in some cases by excitatory responses to microelectrophoretic administration of DL-homocysteic acid), while the remaining 23 were from lung vagal afferents. Most neurons in the former group (87/119) were inspiratory. Out of 96 neurons tested for spinal projections only 14 (12 inspiratory, 2 expiratory) responded antidromically following stimulation at C_3 segment. These results confirm the existence of the DRG in the rat and demonstrate that neurons located in this region have firing patterns generally similar to those previously described in the cat. The main difference is the relative paucity in the rat of neurons projecting spinally below the C_2 level, which indicates that most DRG neurons in this species do not project directly to phrenic and intercostal motoneurons, but to other, as yet unidentified, neuronal groups within the brainstem or upper cervical segments.

Key words: Respiratory neuron; Medulla oblongata; Nucleus of the solitary tract; Extracellular recording; Antidromic stimulation; DL-Homocysteic acid; Vagal afferent

1. Introduction

For many decades adult cats have been used for study of the neural control of breathing. This work has shown that neurons involved in control of respiratory movements are located mainly in the medulla oblongata, where they form two longitudinal columns known as the dorsal respiratory group (DRG) and the ventral respiratory group (VRG). The DRG is located in the dorsomedial medulla, in specific parts of the nucleus of the solitary tract (NTS), while the VRG extends rostrally to the Bötzinger complex and caudally to the spinomedullary junction (for review, see [16]). The ventrally located column has been described in several other species, including rat [17,42,46,47], piglet [28], dog [1,9], monkey [9], guinea pig [44] and rabbit [27]. On the other hand, it remains debatable whether the DRG is a common feature of the respiratory network of all mammalian species.

The DRG was initially identified in the cat [3,4,5,11,32] as a cluster of inspiratory, mainly bulbospinal neurons, located in the ventrolateral subnucleus of the nucleus of the solitary tract (vlNTS). The existence of a similar group of neurons was described in the rabbit [27], guinea pig [44] and fetal sheep [8,26]. An initial extracellular study of this region in the adult rat by Saether et al. [46] reported the presence of a corresponding group of cells (see also [6,35]), a conclusion supported by lesion experiments [24]. However, other extracellular or intracellular studies found few [17] or no [25,52] inspiratory neurons in the NTS and suggested that the DRG is absent in the rat [17,52]. This latter conclusion is in accordance with anatomical tracing studies which demonstrated only a very limited

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projection from the vlNTS to the spinal cord in the rat [39,43,51] (see also [37]). It also corresponds to the results obtained from grafting of peripheral nerves into the medulla, which showed that respiratory units could be recorded from grafts implanted into the ventral medulla, but only infrequently from grafts in the dorsal medulla [21].

In view of this uncertainty, a study was undertaken to re-examine the existence of the DRG in the rat using systematic mapping with extracellular microelectrodes and antidromic stimulation from the spinal cord.

2. Materials and methods

Twelve adult male Wistar rats (400–550 g) were anesthetized with sodium pentobarbital (Nembutal, 80 mg/kg i.p., followed by 4–6 mg/h i.v.), paralysed with pancuronium bromide (Pavulon, 0.4 mg/h) and artificially ventilated with O_2-enriched air after a bilateral pneumothorax (expiratory load, 2–4 cm H_2O). Tracheal pressure (peak, < 10 cm H_2O), arterial blood pressure (mean, > 80 mmHg), end-tidal CO_2 (5–6%) and body temperature (36–37°C) were monitored. The dorsal surface of the medullâ and cervical cord (C_1 to C_7 segments) was exposed. Standard bipolar electrodes were used to record from the central end of a divided phrenic nerve. Bipolar stimulating electrodes were placed on both cervical vagus nerves, which were intact. In addition, monopolar stainless steel stimulating electrodes were inserted bilaterally into the ventrolateral funiculus at the rostral C_3 level.

For extracellular recording glass microelectrodes filled with either 3 M NaCl, 5% Pontamine sky blue in 0.5 M sodium acetate, or 0.15 M DL-homocysteic acid in 0.5 M sodium acetate (resistance, 4–8 MΩ) were used. Recordings were made using an AC amplifier (Neurolog NL 104/125; bandwidth, 10 Hz–6 kHz or 120 Hz–6 kHz) or a DC amplifier (NL 102/125). The latter produced higher noise, but enabled ejection of DL-homocysteic acid through the recording electrode. Units were classified as respiratory if they showed clear on-off type modulation of their firing in phase with cycles of central respiratory activity (as defined by recording from the phrenic nerve) and/or lung inflations (assessed by tracheal pressure). Each respiratory unit was tested for orthodromic response to stimulation of ipsilateral vagus (0.1 ms, 0.2–0.4 V), and for antidromic responses to stimulation of ipsi- and contralateral cervical cord (0.2 ms, up to 1.5 mA). The threshold stimulus intensity for orthodromic activation from the vagus was always compared with the threshold for prolongation of the expiratory phase with trains of stimuli delivered to the vagus at 100 Hz during expiration. The threshold stimulus to elicit this Hering-Breuer type expiratory inhibition in individual animals was 0.1–0.3 V. For testing reponses of respiratory units to single shock vagal stimulation we routinely used a stimulus equal to twice the threshold voltage producing phrenic nerve inhibition after stimulation of the vagus at 100 Hz. Some inspiratory units were further classified as Rα or Rβ [3,4,32] (for discussion of different terminology see [10]) on the basis of their response to maintained lung inflation (4–6 s; 4 to 10 cm H_2O) followed by deflation lasting 2 to 3 s. In addition, short-lasting deflations of the lungs to atmospheric pressure (cf. [31]) were performed when recording from units firing in phase with the ventilator. DL-homocysteic acid was applied by iontophoresis using a continuous negative current of up to 25 nA. Some recording points were marked by iontophoretic injection of Pontamine sky blue (7 μA for 10 min, tip negative). After recording, some ($n = 6$) animals were perfused with a fixative and 75 μm thick coronal sections of the medulla were cut with a Vibratome and counterstained with Neutral red.

3. Results

Tracking was carried out at 75 μm intervals (in both rostrocaudal and mediolateral directions) in the area extending from 1.5 mm rostral to 0.5 mm caudal to obex, from 0.5 to 1.5 mm lateral to the midline and from 0.5 to 1.5 mm below the dorsal medullary surface. Of 142 respiratory units found in this area, 74 fired only with one phase of the central respiratory cycle (69 inspiratory, 5 expiratory), 22 showed a modulation of

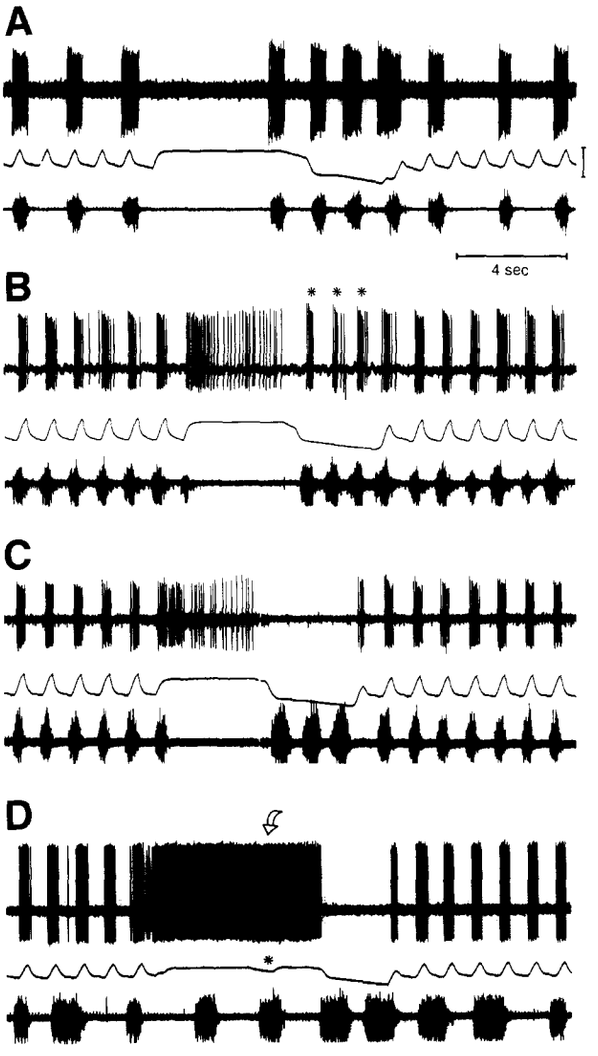

Fig. 1. Examples of recordings from four types of dorsal medullary respiratory units, showing in each case the effects of inflation and deflation maneuvers. Each record (A–D) shows, from top, extracellular unit recording, tracheal pressure (Bar in A = 0–10 cm H_2O) and phrenic nerve activity. A: Rα inspiratory neuron. B: Rβ inspiratory neuron. Asterisks indicate bursts of activity occurring in phase with central inspiration (compare C and D). C: P-cell. D: pulmonary stretch receptor afferent. Note regularity in the frequency of action potentials; a decrease in firing rate in the region indicated by the arrow was observed at an exapanded time-scale when lung inflation was briefly reduced (asterisk).

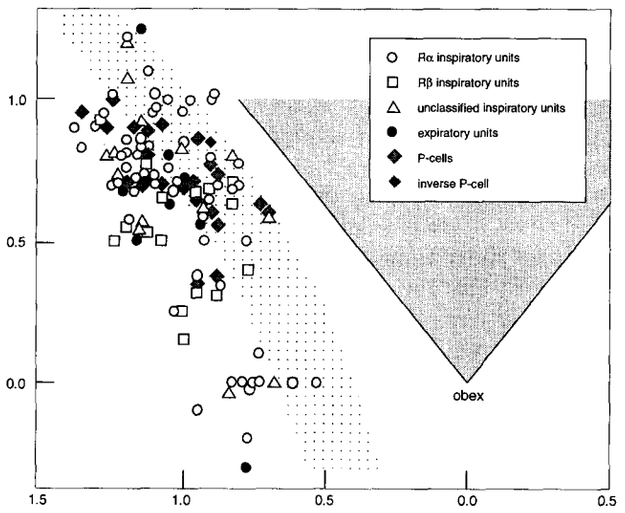

Fig. 2. Locations, based on recording coordinates in relation to obex, of 119 units projected onto one side of dorsal medullary surface. Rostral is above. The gray shape indicates area postrema and the stippled band, the solitary tract.

firing frequencies both in relation to central respiratory rhythm and ventilator-induced or maintained lung inflations and the remaining 46 fired only with changes in tracheal pressure due to the ventilator (38 with lung inflations, 8 with deflations). Examples of frequently observed firing patterns are shown in Fig. 1 and the location of all units ($n = 119$), except primary vagal afferents (see below), in a horizontal projection in Fig. 2.

Fifty-seven units which fired only during neural inspiration and responded to maintained lung inflation performed during expiration in parallel with the phrenic response, were classified as Rα. A typical firing pattern and response to maintained lung inflation is shown in Fig. 1A. Sixteen of these units were mapped in the coronal plane on the basis of Pontamine sky blue deposits. All were located within, or immediately adjacent to, the vlNTS (Fig. 3). Twelve other inspiratory units were not tested with maintained lung inflations and thus were not further classified.

Several distinct types were found among units having combined central respiratory and lung-related inputs ($n = 22$). Sixteen units were excited by ventilator-induced, or larger maintained lung inflations, as well as displaying central inspiratory rhythm (similar to Rβ neurons of Baumgarten and Kanzow [3]; e.g. Fig. 1B). The onset of inspiratory firing in these cells varied from mid to late inspiration (the latter being similar to post-inspiratory neurons of Richter [45]). Two units fired throughout expiration but were inhibited by phasic (ventilator-induced) lung inflations and the remaining four showed miscellaneous patterns (one suppressed in inspiration and excited by lung inflations; one active during expiration and excited during inflation; one active during inspiration and excited during deflation; and one active during expiration and excited by deflation).

Out of 46 units firing only in relation to changes in tracheal pressure, 23 were classified as primary vagal afferents and 23 as pump-related medullary cells (22 as P-cells [4,12] and 1 as an inverse P-cell [41]). Vagal afferents were excited by stimulation of the ipsilateral vagus at a low, sharply defined threshold (0.31 ± 0.1 V, mean $\pm$ S.D.), close to that required to evoke the Hering-Breuer reflex (see section 2). The latency of response ranged from 0.9 to 1.95 ms (1.2 ± 0.3 ms). This

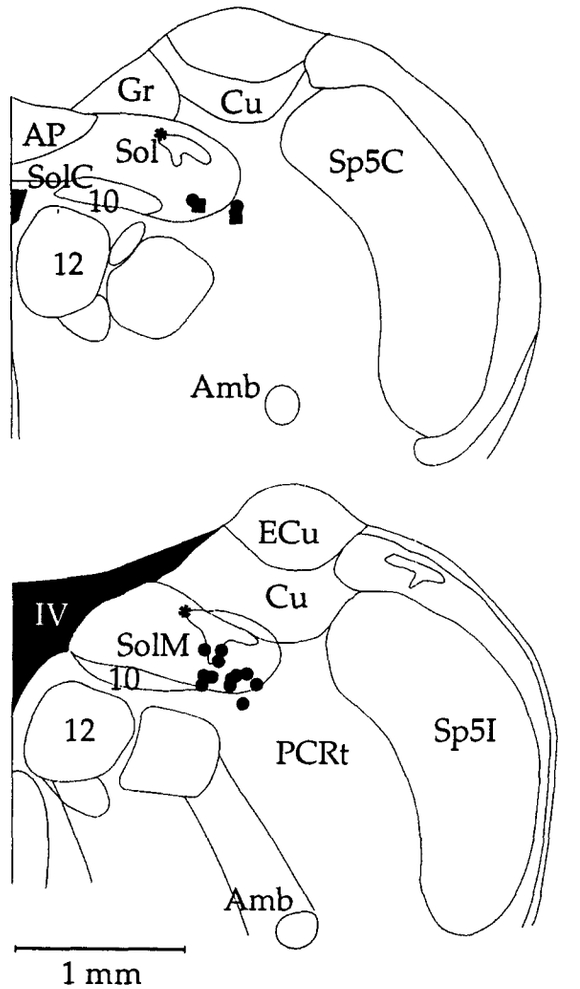

Fig. 3. Location of bulbospinal (filled squares) and non-bulbospinal (filled circles) inspiratory units identified by dye injection at recording sites. Caudal (above) and rostral (below) planes adapted from atlas of Paxinos, G. and Watson, C. (*The Rat Brain in Stereotaxic Coordinates* (1986) Academic Press, North Ryde, Australia). Asterisks, reference points for histological measurements. *Abbreviations:* Amb, ambiguus nucleus; AP, area postrema; Cu, cuneate nucleus; ECu, external cuneate nucleus; Gr, gracile nucleus; PCRt, parvocellular reticular nucleus; Sol, solitary tract; SolC, nucleus of the solitary tract, commissural part; SolM, nucleus of the solitary tract, medial part; Sp5C, spinal trigeminal nucleus, caudal part; Sp5I, spinal trigeminal nucleus, interpolar part; IV, fourth ventricle; 10, dorsal motor nucleus of vagus; 12, hypoglossal nucleus.

latency was constant for each individual unit, irrespective of the stimulus intensity (1–5 times threshold). Sixteen of these units had inflation-related activity (from pulmonary stretch receptors, as demonstrated by little or no adaptation following maintained inflation; Fig. 1D), while 7 fired during deflations. The latter units were regarded as axons from rapidly adapting lung receptors (cf. [31]). Inflation-related units responded to ipsilateral vagus stimulation with a latency of 1.2 ± 0.3 ms and threshold 0.33 ± 0.1 V; deflation-related units with a latency 1.2 ± 0.5 ms and threshold 0.25 ± 0.1 V (means $\pm$ S.D.).

Twenty-two units which were identified as P-cells fired during each inflation cycle of the ventilator and during maintained lung inflations, but not with the central inspiratory rhythm (even when the central inspiratory activity was increased following deflation of the lungs, Fig. 1C). One unit fired in time with ventilator-induced lung *deflations* and was described as an 'inverse P-cell' (this unit was inhibited by stimulation of the ipsilateral vagus with a latency of 4 ms). Although the pattern of P-cell activity was similar to that observed in afferents from pulmonary stretch receptors (see above), P-cells could be distinguished from pulmonary afferents by the following criteria: (1) the latency of their excitatory response to vagal stimulation was generally longer than that of afferents from lung stretch receptors (1.9 ± 0.5 ms); (2) responses were evoked at generally higher thresholds (0.47 ± 0.3 V); (3) during suprathreshold stimulation, latency jitter was usually observed, ranging from 0.2–0.5 ms; (4) unlike the response of afferents from slowly adapting stretch receptors which fired tonically and 'regularly' during maintained lung inflations (Fig. 1D), P-cells responded to maintained inflations in an irregular fashion and displayed a variable but significant amount of adaptation (Fig. 1C).

In addition to primary vagal afferents and P-cells, other types of units examined were excited by ipsilateral vagal stimulation. These responses were observed in 12 of 57 Rα units (latency, 2.5 ± 0.2 ms) and 11 of 16 Rβ-type units (latency, 3.4 ± 1.5 ms).

Fourteen out of 96 tested units could be activated antidromically [29] from the cervical cord (Fig. 4). Most of these (11/14) fired during inspiration and were inhibited by maintained lung inflations (Rα cells, Fig. 4). The antidromic responses (latency, 1.9 ± 0.9 ms, mean $\pm$ S.D.) were evoked from the contralateral half of the spinal cord ($n = 7$), ipsilateral half ($n = 2$), or both ($n = 1$, Fig. 4B,C). In one case laterality could not be determined. The conduction velocity, calculated from single point stimulation and assuming a linear axonal trajectory, was 4–12 m/s (7.4 ± 3.1 m/s). In addition, two units which showed activity during expiration but were inhibited by lung inflation and one active during inspiration and excited by lung deflations, projected to the cord. None of the Rβ-type units tested ($n = 11$) was shown to be bulbospinal.

Sixteen units were tested with microelectrophoretic administration of DL-homocysteic acid. Fourteen (9 Rα, 2 Rβ, 2 P-cells, 1 expiratory unit) responded with an increase in firing rate and a decrease in amplitude.

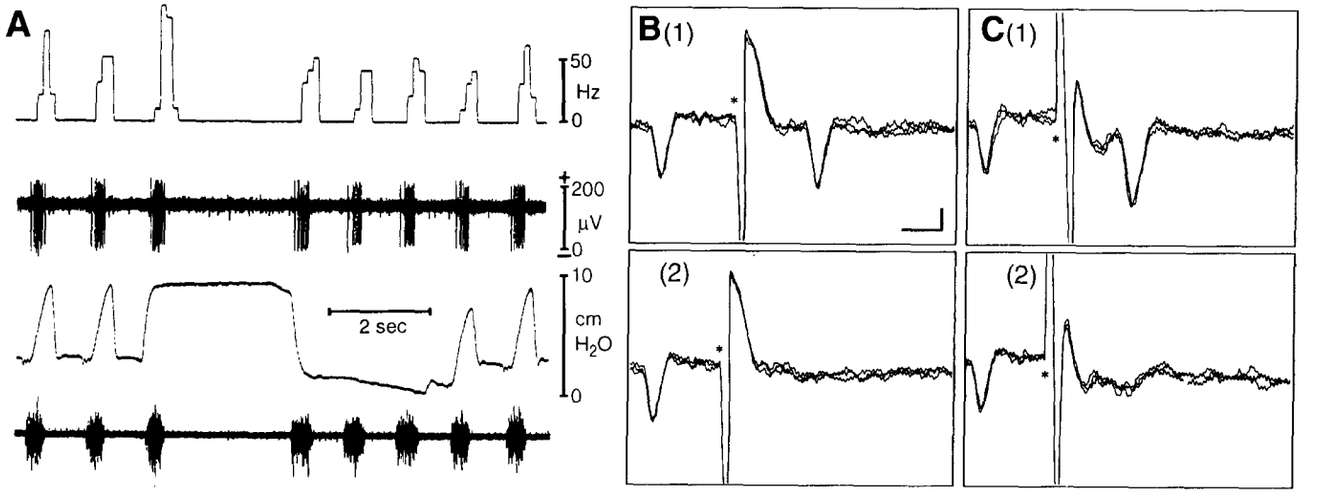

Fig. 4. Features of a bulbospinal Rα neuron. A: recording showing effect of lung inflation and deflation. Traces from top; spike count, extracellular potentials, tracheal pressure, phrenic nerve activity. B and C: collision tests with ipsilateral ($B_{(1),(2)}$) and contralateral ($C_{(1),(2)}$) stimulation at C_3 segment. Stimulus artifact marked by asterisks. Bar = 1 ms, 50 mV.

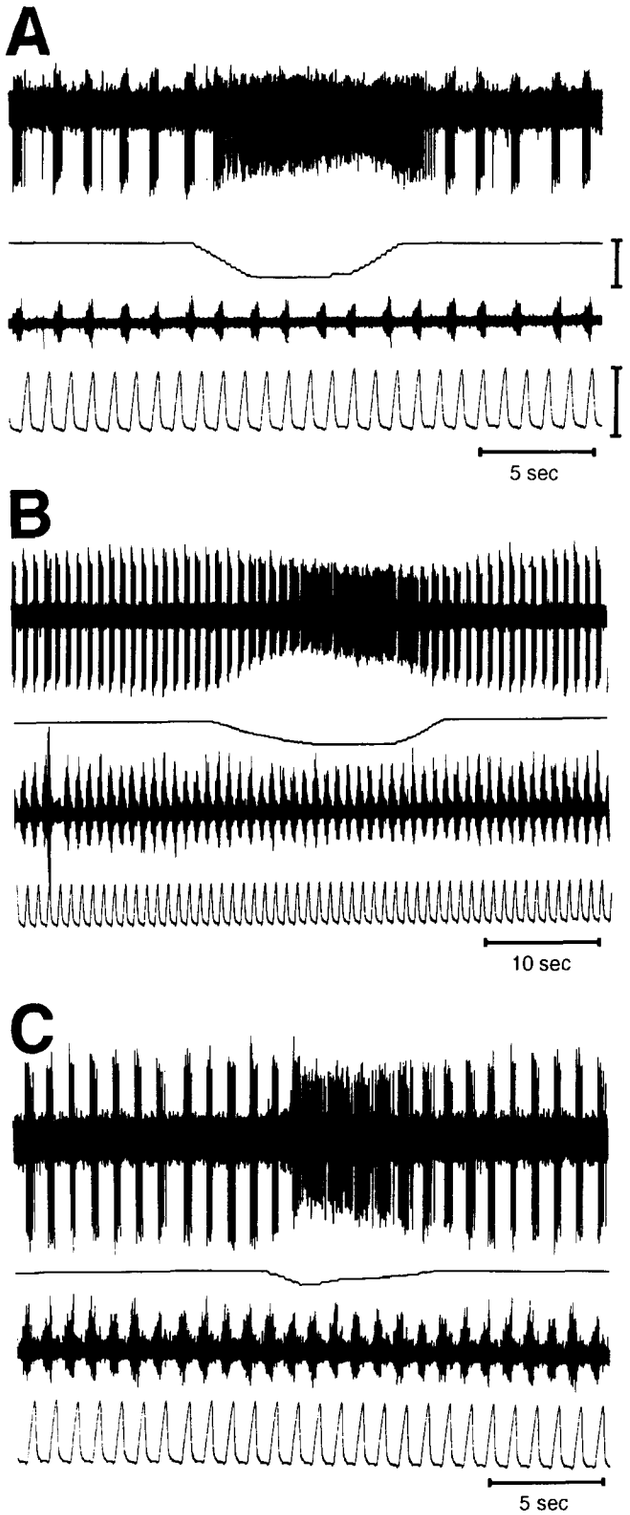

Fig. 5. Examples of excitatory responses of three DRG neurons to microelectrophoretic application of DL-homocysteic acid. Traces from above: unit activity, current (calibration bar in A, 50 nA), phrenic nerve discharge and tracheal pressure (calibration bar in A, 0–10 cm H_2O). A: Rα cell. B: Rβ cell. C: P-cell.

Examples are shown in Fig. 5. Two remaining units which were not excited fulfilled previously listed criteria for vagal axons.

4. Discussion

These results indicate a concentration of respiratory neurons in the dorsomedial part of the medulla oblongata of the rat corresponding to the DRG previously described in the cat and thus generally confirm the conclusion reached by Saether et al. [46]. One difference between our result and that obtained by those authors is that respiratory units recorded in our study were found in a circumscribed region (vlNTS and immediately adjacent reticular formation) while recording sites in the other study [46] were more scattered, with little separation between DRG and VRG regions. The difference may be due to barbiturate anesthesia used in the present study, as opposed to ketamine used by Saether et al. [46]. It has been suggested [36] that barbiturates 'simplify' the respiratory control system by eliminating all activity except that which is essential for maintaining respiratory motor output.

It is not clear why few [17] or no [25] respiratory units were reported in this region by other authors. One reason may be the small size of NTS neurons, indicated (at least for those projecting to the spinal cord) by their slow conduction velocity (mean, 7.4 m/s; cf. also a study of Portillo and Núñez-Abades [43] who showed spinally projecting NTS cells less than 10 μm in diameter). Such small cells generally require for extracellular recording more selective, higher impedance microelectrodes; those used in our study had substantially higher impedance (4–8 MΩ) than the electrodes used by Ezure et al. [17] (1–2 MΩ). The importance of microelectrode configuration was also noted in a recent extracellular study of DRG neurons in the guinea pig [44]. The small size of DRG neurons in the rat could also explain the negative conclusion of the intracellular study of Zheng et al. [52]. These authors found that of 248 medullary respiratory neurons impaled, only three were in the dorsal medulla and all three were hypoglossal motoneurons. Thus intracellular studies of DRG neurons in the rat may not be feasible in vivo and alternative techniques may be needed, such as intracellular recordings from slices [eg. 19] or from cultured NTS neurons of neonatal rats ([50]; cf. also [18]). However, such in vitro studies usually face the problem of functional identification of examined neurons.

It has been argued that respiratory activity recorded in the dorsomedial medulla of the rat with extracellular microelectrodes originates from passing axons, such as those of inspiratory motoneurons of nucleus ambiguus, rather than from cell bodies of DRG neurons [24,35,52].

Axon collaterals of expiratory Bötzinger neurons have been traced to this region [7], as have collaterals of inspiratory VRG neurons [33]; cf. also results of anterograde tracing studies [15,51] and some unidentified inspiratory axons have been impaled in this region [52]. Moreover, some investigators [25,51] described respiratory (inspiratory) 'noise' when recording from this region, which could be interpreted as recording from axons rather than cell bodies. We could not exclude the possibility that some of our recordings were from axons (in fact, we could easily record action potentials from primary vagal afferents). However, we believe that most of our recordings were made from cell bodies, as all 14 units which were classified as 'somal' on the basis of standard electrophysiological criteria, responded with excitation when tested with DL-homocysteic acid. This is consistent with an excitatory effect of this amino acid on the soma-dendritic region, in contrast to the lack of effect on axons [20].

The final two points concern 'composition' of the DRG and axonal projections of these neurons. The types of activity recorded in our study were generally similar to those previously reported in the DRG of the cat [2–5,32]. The majority of the neurons were classified, using previously established criteria, as Rα inspiratory, Rβ inspiratory, P-cells, or expiratory cells, with relatively few showing miscellaneous activity patterns. As many neurons showed central respiratory activity and were also affected by input from lung afferents, it is suggested that the DRG neurons in the rat, like those in the cat, are mainly responsible for the integration of peripheral sensory input with centrally generated patterns. However, it must be mentioned that the classification protocol used in our study did not include the important withholding-inflation test which can be used in animals ventilated with a cycle-triggered ventilator (eg. [10]). Therefore a more detailed classification of DRG neurons in the rat awaits further studies.

With respect to axonal projections, there seems to be a clear difference between cats and rats. In the cat the majority of DRG neurons, both Rα and Rβ, are bulbospinal (e.g. [4,32]), and often have a direct excitatory input to phrenic and intercostal motoneurons (e.g. [10,11,14,32]). Some axon collaterals of these neurons were also identified in the medulla [40,49], but their synaptic targets are not known (cf. also [13]). In the rat, we observed relatively few NTS neurons which projected below the C_2 segment, although the possibility that they projected to upper cervical inspiratory neurons in the C_1 and C_2 segments [30] was not tested. Only 14% of tested inspiratory cells responded antidromically from the C_3 segment, compared with approximately 80% of bulbospinal cells in the DRG of the cat [4]. This low proportion of electrophysiologically identified respiratory bulbospinal units in the rat, as compared with the cat, also agrees with previous anatomical studies. In the cat, a strong projection from the vlNTS to the spinal cord has been described with both anterograde and retrograde tracing and degeneration studies [34,38,48]. This contrasts with results obtained in rats. In the anterograde tracing studies in the rat a few labelled axons were found in the cervical cord, but none in the phrenic nucleus [51]. In a retrograde study in this species by Onai et al. [39] only approximately 1% of all retrogradely labelled medullary neurons from the phrenic nucleus were found in the NTS, while a somewhat larger proportion (4%), including some double-labelled cells from bilateral phrenic injections, was described in the study by Portillo and Núñez-Abades [43]. NTS cells retrogradely labelled following injections to the phrenic nucleus, or upper and middle segments of the thoracic spinal cord, were also found in other studies [22,23], but no counts of labelled cells were reported.

In conclusion, these results confirm the existence of the DRG in the rat. However, these neurons appear to play only a small role in the direct control of spinal respiratory motoneurons through direct bulbospinal pathways. They are likely to control other respiratory neurons in the brainstem through local (non-spinally projecting) axon collaterals and ultimately respiratory motoneurons through polysynaptic pathways involving other medullary neurons. Further studies are necessary to elucidate the functional role of these neurons in the rat.

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