Electrophysiological Properties of Phrenic Motoneurons in Adult Rats

Fumiaki HAYASHI and Yasuichiro FUKUDA

Department of Physiology, School of Medicine, Chiba University, Chuou-ku, Chiba, 260 Japan

Abstract Electrophysiological properties of phrenic motoneurons (PMs) were studied by intracellular recordings in anesthetized, paralyzed, and artificially ventilated adult rats. Our results revealed that rat PMs, as compared to cat PMs, had a shorter afterhyperpolarization duration (55±13 ms; mean±SD), a narrower half-width of action potential, a larger membrane input resistance ($R_m$; 2.0±0.6 MΩ), a smaller rheobase ($I_{th}$) and a shorter minimum paired pulse interval to provoke the second spike (1.9±0.6 ms). These features indicate that the rat PMs possess higher frequency responsiveness than cat PMs. Based on the time of firing onset relative to the onset of whole phrenic nerve activity (relative onset time; ROT) or on the absence of discharge during inspiration, the PMs could be classified into four types: early recruited with $\text{ROT}<10.0\%$; late recruited with $12.5\%<\text{ROT}<37.5\%$; very late recruited with $\text{ROT}>45\%$, and quiescent which was not recruited during normal experimental conditions. A number of differences in the membrane properties such as end-expiratory membrane potential, $R_m$, and $I_{th}$ among the four cell types are discussed in relation to the recruitment order. Inhibitory postsynaptic potentials (IPSPs) were present during the late expiratory phase (stage II expiration) in all PMs tested.

Key words: phrenic motoneurons, intracellular recording, adult rat, recruitment order, membrane properties.

Phrenic motoneurons (PMs) integrate a number of afferent inputs and provide rhythmic inspiratory drive to the diaphragm, a main respiratory pump muscle. The discharge pattern of PMs in response to input signals from the medullary centers depends in part upon their electrophysiological properties, e.g., membrane input resistance ($R_m$), rheobase ($I_{th}$), and afterhyperpolarization duration (AHP$_{dur}$). Although the intrinsic properties of PMs are well explored in the cat [1–5], there has been no study on the PMs in other species such as the rat. The rat is becoming a standard experimental animal for studying the neuronal components controlling

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respiratory function [6–8]. In addition to differences in the breathing pattern between the rat and cat, there is a controversy regarding similarities of the organization of medullary respiratory neurons between them [8]. Using in vitro preparations, Smith et al. [9] and Liu et al. [10] have reported electrical properties of PMs in the newborn rat. However, direct extrapolation from the properties of PMs obtained in the in vitro newborn rat to those in the adult rat may be unreliable because of differences in maturation and experimental conditions. In the present study, we have analyzed the membrane properties of PMs of adults rats in vivo, and have compared them with those reported previously for cats [1–5] and for neonatal rats [9].

Henneman’s size principle [11] states that the recruitment order of sacro-lumbar motoneurons is determined by their intrinsic properties (i.e., $R_m$, $I_m$, $AHP_{dur}$, conduction velocity). The good correlation found between the recruitment order in the diaphragmatic motor unit (MU) of the cat and the conduction velocities in the phrenic nerve [12] indicates that the size principle applies to the phrenic-diaphragmatic MU. Our attempt has thus been to classify PMs according to the recruitment order on their possible differences in the electrical properties.

MATERIALS AND METHODS

General procedure. Twenty-five young adult male Wistar rats (weight: 320–520 g) were anesthetized with urethane (1.5 g/kg, i.p.) or pentobarbital sodium (60 mg/kg, i.p.). Throughout the experiments, adequate level of anesthesia was assessed by stability in arterial blood pressure or heart rate against noxious stimuli. Supplementary doses of anesthetics (10–20% of the initial dose, i.v. or i.a.) were injected when necessary. Care and treatment of animals were performed according to the “Guiding principles for the care and use of animals in the field of physiological sciences” of the Physiological Society of Japan. The femoral artery and vein were cannulated to measure the arterial blood pressure and to administer drugs, respectively. Bicarbonate saline (Na$^+$ 154 mM, Cl$^-$ 124 mM, HCO$_3^-$ 30 mM) was infused intermittently (approximately 5 ml/h) to maintain circulatory stability. The animal was artificially ventilated (frequency: 70–90 breaths/min, tidal volume: 2.5–4.0 ml) with positive end-expiratory pressure of 2–4 cmH$_2$O. Both vagi were cut in the neck. The animal was paralyzed initially with pancuronium bromide (1 mg, i.v.) and an additional dose (40–100% of the initial dose) was given every hour. Tracheal CO$_2$ was continuously monitored with an infrared CO$_2$ analyzer (NEC San-ei, 1H26). The end-tidal CO$_2$ concentration was maintained at 6–7% by adding CO$_2$ to a hyperoxic ($F_{O_2}=0.35–0.5$) inspired gas mixture. In some experiments, hypocapnic apnea was attained by cutting off the supply of CO$_2$ to the inspired gas. The animal was suspended in a stereotaxic head-holder and a spinal frame using vertebral clamps placed at T$_1$ and L$_2$. The cervical spinal cord was exposed by laminectomy from C$_1$ to C$_7$. The pia mater at the recording sites was removed with fine forceps. The fourth cervical branch (C$_4$) of the left or both

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phrenic nerves was isolated in the neck by a dorsolateral approach, cut distally and mounted on silver bipolar electrodes for either recording or antidromic stimulation. In some animals the left thoracic phrenic nerve was placed inside a coiled silver wire electrode for stimulation. The coiled electrode was immersed in a Vaseline-mineral oil mixture, and was covered with a polyethylene tubing to isolate the nerve from surrounding tissues. The exposed nerve and spinal cord were covered with warm paraffin oil. Rectal temperature was maintained at 35–37°C by means of external heating lamps.

Stimulation and recording. Phrenic nerve mass discharge was recorded from the right or left phrenic nerve in the neck, amplified (band-pass: 0.1 to 3 kHz), full-wave rectified, and integrated (time constant: 20 ms). A glass microelectrode (DC resistance: 8–20 MΩ) was usually filled with 2 m K-citrate solution but in two cases with 3 m KCl solution. The microelectrode was advanced with an electronic micromanipulator (Narishige, MO-81). PMs were intracellularly recorded in C7–C8 regions from 0.1 mm medial to 0.3 mm lateral to the dorsal root entry zone, and at a depth of 1.3–2.0 mm from the surface. As a reference, a silver-silver chloride wire was inserted into the neck muscle. Suprathreshold antidromic stimulation to PMs ranged from 2 to 10 V (duration: 0.1 ms, 2 Hz). Spontaneous spikes of PMs were displayed on a storage oscilloscope, recorded on a thermal array recorder, and stored on magnetic tape. PMs were identified antidromically from the phrenic nerve. These neurons showed a depolarizing shift of membrane potential (central respiratory drive potential; CRDP) during inspiration (phrenic nerve mass discharge). The neurons which demonstrated CRDP but could not be antidromically activated were regarded as segmental interneurons.

Experimental protocol and data analysis. All electrophysiological measurements of the membrane properties of PMs were made during the expiratory phase. The resting membrane potential (\(V_m\)) was measured as the difference between intracellular and extracellular potentials. The extracellular potential was taken in the vicinity of a neuron after the electrode was withdrawn from the cell. Analyses were made on the cells with \(V_m\) of at least \(-55\) mV and an action potential of 50 mV. To quantify the recruitment order, the relative onset times (ROTs) were calculated as the delay from the initiation of whole phrenic discharge to the first spike divided by the inspiratory time (\(T_i\)) and was expressed as a percentage. The CRDP was measured as the voltage difference between end-expiration and the onset of the first spike (or a maximum potential if there was no spike). The firing threshold (\(V_{th}\)) was defined as the membrane potential at which the first action potential was generated in the spontaneous discharge. The average firing frequency (mean \(f\)) during inspiration was assessed from the number of spikes in the bursts divided by the burst duration. These values were averaged for 5 consecutive breaths.

The action potential height (APH), half-width of the spike (APW₁₂), after-hyperpolarization duration (AHP₉₀), and the maximum paired-pulse interval (MPPI) capable of blocking the second of two elicited spikes were measured from
the antidromic spikes evoked during a late part of expiration. The APH was measured from the pre-stimulus membrane potential to the peak of the spike. The APW/1/2 was defined as the duration at half of the APH. The AHP_{dur} was measured from the spike onset to a point where V_m returned to the pre-stimulus level.

The input resistance (R_m) was determined by two methods. The first technique was to pass hyperpolarizing current pulses (50 ms duration, -2 to -10 nA) with at least two different intensities to measure the resultant displacement of membrane potentials. When a good bridge balance was not attained, the data obtained by this method was rejected. The second technique was to apply hyperpolarizing pulses (more than 100 ms duration) with antidromic action potentials evoked at least 100 ms after the start of the pulses [1-5, 13]. The R_m was calculated from plots of the current versus the spike height. The rheobase (I_{rhe}) was defined as the magnitude of a depolarizing current pulse (100-500 ms) capable of eliciting spikes in 50% of trials.

To identify inhibitory post-synaptic potentials (IPSPs), we attempted to reverse the post-synaptic hyperpolarization to depolarizations by intracellular injection of negative currents or by chloride iontophoresis (up to 35 nA, 5-20 min) using a KCl-filled electrode.

The data were analyzed on a Macintosh computer using the Statview II statistical program. Statistical analysis included a single factor analysis of variance of data grouped according to the cell type (recruitment order). Each parameter from the data (Table 1) is presented as the mean±standard deviation (SD). Differences were considered to be significant if p < 0.05.

RESULTS

Recruitment order of phrenic motoneurons

We recorded intracellularly from 90 PMs within the C_4 and C_3 segments of the spinal cord. Figure 1 shows the distribution of ROTs of the cells that discharged in inspiration. Four categories were defined. The first group (n=21, 23%) had ROTs of less than 10% (early recruited type; type E cell; Figs. 1 and 2A). The second group (n=22, 24%) were late recruited cells (type L cell; Figs. 1, 3A) with ROTs between 12.5 and 37.5%. The third group (n=16, 18%) were recruited very late (type VL cell; ROT > 45%, Figs. 1 and 4); in some of these cells, firing did not occur in every cycle. Out of 90 cells, 31 did not discharge spontaneously (quiescent type; type Q cell; 34%; Fig. 5A).

Electrical properties of phrenic motoneurons

Antidromic latencies from stimulation of the phrenic nerve in the neck (AL-n) ranged from 0.3 to 0.7 ms (n=75) and from stimulation in the thorax (AL-t) from 1.5 to 2.7 ms (n=15) (Table 1). Depolarizing afterpotentials were observed in some cells, especially in the late and quiescent types (Figs. 3C and 5B). With decreasing intervals between paired pulses, the second spike developed a prominent

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Fig. 1. Distribution of ROT in relation to the initiation of whole phrenic nerve activity for 59 active PMs. The arrows indicate discontinuities in the distribution. N, number of observations.

Fig. 2. A: Upper trace, membrane potential of a type E cell ($V_m$); lower trace, integrated phrenic nerve activity ($f_{Phr}$). B: Following injection of hyperpolarizing current ($-20\,\text{nA}$ for $12\,\text{min}$), the $V_m$ trajectory during stage II expiration was reversed to a depolarizing direction. C and D: Antidromic spikes displayed at two different time scales. The arrow indicates the point at which the ascending part of the AHP trajectory returned to the pre-stimulus level.

inflexion in its rising phase; then the somato-dendritic spike disappeared (Fig. 5B). In 85 of 90 cells (94%), a spike overshoot (OS, $>0\,\text{mV}$) was exhibited in the action potential (Table 1). The end-expiratory membrane potentials ($E_m$), AHP$_{dur}$ (Fig.
Fig. 3. A and B: Upper trace, $V_m$ of a late recruited (type L) cell; lower trace, $f$Phr. Action potentials are clipped. B: Injection of hyperpolarizing current ($-35 \text{ nA for 12 min}$) revealed reversed IPSPs during expiration. C: Antidromic action potential with a depolarizing afterpotential.

Fig. 4. $V_m$ (upper trace) and $f$Phr (lower trace) of a type VL cell. Note that spikes did not occur in each cycle.

2D), APW$_{1/2}$, and APH averaged $-67 \text{ mV}$, $55 \text{ ms}$, $0.69 \text{ ms}$, and $77 \text{ mV}$, respectively (Table 1). CRDP was observed in all cells and ranged from 2 to 20 mV ($n=90$, Table 1 and Fig. 6). The $V_{\text{th}}$ ranged between $-47$ and $-62 \text{ mV}$ ($n=59$, Table 1).

Figure 6 illustrates the frequency distributions of $E_m$, $AHP_{\text{dur}}$, APW$_{1/2}$, $R_m$, $I_{\text{rh}}$, and CRDP. The $E_m$, $R_m$, and CRDP appear to be distributed unimodally, but $AHP_{\text{dur}}$, APW$_{1/2}$, and $I_{\text{rh}}$ do not (Fig. 6).

There were a number of differences in electrical properties among the four cell types, as summarized in Table 1. $R_m$ was significantly lower ($p<0.01$) in types L
and Q than in type E. $E_m$ was significantly lower in types E or L than in types VL or Q ($p < 0.01$). $I_{in}$ was highest in type Q ($p < 0.01$ or $p < 0.05$), and higher in type VL cell than in type E ($p < 0.05$). There was a significant difference in $V_{th}$ between types E and VL cells ($p < 0.01$). CRDP was significantly larger in type VL than in types L or E ($p < 0.01$), and smallest in type Q ($p < 0.01$).

AL-n and AL-t were longer in type E than in the other types ($p < 0.01$ or $p < 0.05$), although the difference in AL-n between types E and VL was not statistically significant. The average spike frequency (mean $f$) was higher in type E than in L ($p < 0.01$). Even for the parameters with significant differences among cell types (Table 1), the distribution histograms reveal considerable overlaps among them (Fig. 6).

Correlation of $E_m$, $V_{th}$, $R_m$, and CRDP with ROT was examined to assess which variables may determine ROT (Fig. 7). Significant correlations of ROTs with $E_m$ ($r = -0.57, p < 0.01$), $V_{th}$ ($r = -0.35, p < 0.05$), and CRDP ($r = 0.58, p < 0.01$) were found, but not with $R_m$. There was a significant correlation between AL-n and $R_m$ ($r = 0.33, p < 0.05$).

**IPSPs during expiration**

Chloride iontophoresis or membrane hyperpolarization revealed chloride-dependent IPSPs in 18 cells (type E, 11 cells; type L, 7 cells). In these cells, an injection of a hyperpolarizing current step reversed the continuous hyperpolarizing waves during the late expiratory phase (stage II expiration [14]) to marked depolarizing waves (Figs. 2B and 3B). Usually a stronger and longer hyperpolarizing current was required to reverse the IPSPs in type L cells. In type VL and Q cells, the reversal of IPSPs was less prominent and could not be detected in some cells.
Table 1. Comparison of electrical properties of rat phrenic motoneurons among different cell types based on recruitment order.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Type E</th>
<th>Type L</th>
<th>Type VL</th>
<th>Type Q</th>
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<tr>
<td>$E_m$ (mV)</td>
<td>−67±7</td>
<td>−62±4</td>
<td>−64±6</td>
<td>−71±7</td>
<td>−71±7</td>
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<tr>
<td></td>
<td>(90)</td>
<td>(21)</td>
<td>(22)</td>
<td>(16) OCH</td>
<td>(31) O++</td>
</tr>
<tr>
<td>AHPdur (ms)</td>
<td>55±13</td>
<td>60±11</td>
<td>60±17</td>
<td>51±7</td>
<td>50±13</td>
</tr>
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<td></td>
<td>(44)</td>
<td>(12)</td>
<td>(8)</td>
<td>(9)</td>
<td>(15)</td>
</tr>
<tr>
<td>APWt2 (ms)</td>
<td>0.69±0.23</td>
<td>0.75±0.22</td>
<td>0.74±0.22</td>
<td>0.64±0.26</td>
<td>0.64±0.22</td>
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<tr>
<td></td>
<td>(90)</td>
<td>(21)</td>
<td>(22)</td>
<td>(16)</td>
<td>(31)</td>
</tr>
<tr>
<td>APH (mV)</td>
<td>77±9</td>
<td>70±7</td>
<td>73±8</td>
<td>83±9</td>
<td>80±8</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(21)</td>
<td>(22)</td>
<td>(16) OCH</td>
<td>(31) O+++</td>
</tr>
<tr>
<td>OS (mV)$^{1)}$</td>
<td>9±5</td>
<td>8±6</td>
<td>8±6</td>
<td>12±5</td>
<td>9±5</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(21)</td>
<td>(22)</td>
<td>(16)</td>
<td>(31)</td>
</tr>
<tr>
<td>$V_{th}$ (mV)</td>
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<td>−54±4</td>
<td>−56±5</td>
<td>−58±4</td>
<td>N/A</td>
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<tr>
<td></td>
<td>(59)</td>
<td>(21)</td>
<td>(22)</td>
<td>(16) OCO</td>
<td>(31) O++</td>
</tr>
<tr>
<td>CRDP (mV)</td>
<td>8±4</td>
<td>8±3</td>
<td>9±3</td>
<td>14±4</td>
<td>4±2</td>
</tr>
<tr>
<td></td>
<td>(90)</td>
<td>(21)</td>
<td>(22)</td>
<td>(16) OCH</td>
<td>(31) O++</td>
</tr>
<tr>
<td>$R_m$ (MΩ)</td>
<td>2.0±0.6</td>
<td>2.5±0.7</td>
<td>1.7±0.6</td>
<td>2.0±0.6</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td></td>
<td>(51)</td>
<td>(11)</td>
<td>(11)**</td>
<td>(10)</td>
<td>(19)</td>
</tr>
<tr>
<td>$I_{th}$ (nA)</td>
<td>7.7±4.1</td>
<td>2.8±1.3</td>
<td>4.8±2.5</td>
<td>7.2±5.0</td>
<td>10.5±2.3</td>
</tr>
<tr>
<td></td>
<td>(28)</td>
<td>(5)</td>
<td>(4)</td>
<td>(5) OCO</td>
<td>(14) O++</td>
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<tr>
<td>AL-n (ms)</td>
<td>0.47±0.09</td>
<td>0.52±0.09</td>
<td>0.46±0.08</td>
<td>0.47±0.07</td>
<td>0.45±0.09</td>
</tr>
<tr>
<td></td>
<td>(75)</td>
<td>(16)</td>
<td>(17)**</td>
<td>(15)</td>
<td>(27)</td>
</tr>
<tr>
<td>AL-t (ms)</td>
<td>2.0±0.3</td>
<td>2.4±0.2</td>
<td>1.9±0.2</td>
<td>1.7</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td></td>
<td>(15)</td>
<td>(5)</td>
<td>(5)**</td>
<td>(1) OCO</td>
<td>(4) OCO</td>
</tr>
<tr>
<td>mean f (Hz)</td>
<td>43±16</td>
<td>55±14</td>
<td>33±10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(9)</td>
<td>(11)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPPI (ms)</td>
<td>1.9±0.6</td>
<td>1.9±0.7</td>
<td>2.0±0.6</td>
<td>1.9±0.4</td>
<td>2.0±0.7</td>
</tr>
<tr>
<td></td>
<td>(31)</td>
<td>(5)</td>
<td>(5)</td>
<td>(7)</td>
<td>(14)</td>
</tr>
</tbody>
</table>

The values in the parentheses indicate the number of observations. Mean f, mean spike frequency. *Significant differences ($p<0.05$ and $p<0.01$) between type L and type E; ** between type VL and type E ($p<0.01$) or type L ($p<0.01$); + between type Q and type E, type L, or type VL ($p<0.01$); ° between type Q and type VL ($p<0.05$). N/A, data is not available. $^{1)}$ Data include 5 cells which did not exhibit the overshoot.

**Expiratory related neurons within phrenic nucleus**

We could record a total of seven neurons with expiratory-related activity and hyperpolarization during inspiration. These cells were not antidromically activated from the ipsilateral phrenic nerve, and were presumed to be segmental interneurons. These cells were usually encountered slightly dorsal to or in the dorsal part of the phrenic nucleus (1.1–1.5 mm depth) where an antidromic field potential could be seen. The discharge rate was fairly constant during expiration (continuously discharging expiratory cells), and CRDP ranged from 5 to 16 mV. Cells showing post-inspiratory discharges [14] were not encountered. Hypocapnic apnea

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introduced by lowering the level of CO₂ in the inspired gas led to cessation of the synchrony of the hyperpolarizing potentials with inspiration and to slight hyperpolarization of the $V_m$ accompanied by synaptic noise (Fig. 8).

DISCUSSION

This is the first in vivo study to use intracellular recordings to establish the electrical properties of PMs in adult rats. We thus revealed differences in the electrical properties between rat and cat PMs. Our results also showed that the electrical properties of the rat PMs differ among their subtypes classified by the recruitment order.

According to ROT in rat PMs, we defined types E, L, VL, and Q. Dick et al. [12] reported that cat PMs were recruited during spontaneous breathing in an order related to their axonal conduction velocities. From data on the relationship between the conduction velocity and the input membrane resistance of lumbar motoneurons [11, 15], Dick et al. concluded that the size principle could predict the recruitment order of diaphragmatic motor units [12]. As we did in this study, many investigators have assessed the recruitment order of naturally discharging PMs according to ROT [2-5, 12].

Kong and Berger [16] have determined the recruitment order of rat PMs by means of single axonal recordings, and defined type E and L cells with ROTs of less than 12.5% and between 15 and 42.5%, respectively. They reported that the percentages of E and L cell fibers were 58 and 42%, respectively. This ratio of type E and L cells was similar to our result (E vs. L; 21 vs. 22 cells). No fibers with
ROTs greater than 42.5% were encountered in their study [16]. It would be hard with their technique to identify quiescent fibers (cells) or their experiments might have been conducted in a slightly hypocapnic condition (cf. Fig. 8), whereas we monitored the level of end-tidal CO₂ (43-50 mmHg) within the normal or moderately hypercapnic range for anesthetized rats [17].

Type Q cells had a relatively low $R_m$ and a high $E_m$. These factors may contribute to the highest $I_{th}$ (Table 1). The high $I_{th}$ and a small CRDP may explain why these neurons remain quiescent even in the presence of a respiratory input.
These cells remain inactive in the cat [3] and rat (unpublished observations) even during moderate hypercapnia. Type Q cells would be activated only during non-respiratory motor movements (i.e., aspiration reflex, vomiting, coughing, etc.) [4, 18], and therefore provide an “emergency reserve” for increasing diaphragmatic force [4].

Type VL cells had a high $E_m$, a large CRDP, and a high $I_{th}$. A combination of these properties may cause discharges with a late recruitment. Regarding the differentiation of type L and E cells, Kong and Berger [16] emphasized that in response to CO$_2$ stimulation, the ROT of L type axons did not shift to the ROT range of E cells ($<12.5\%$). In our study, compared to type L cells, E cells had a larger $R_m$, a lower $E_m$, a lower $I_{th}$, and a longer antidromic latency. Type E cells might thus reach $V_{th}$ more effectively than type L cells in response to a similar synaptic input.

Antidromic latencies were longer in type E than in the other types and a significant correlation was seen between AL-n and $R_m$. Type E cells may thus be small cells with high $R_m$ and slowly conducting thin axons [19]. This idea is partly supported by the morphological evidence that total cell surface areas are larger in type Q cells than in type E and L cells [20]. There was no major difference in AHP$_{dur}$ among the cell types. In this regard, Jodkowski et al. [4] reported that AHP$_{dur}$ is not different between active and non-active cells.

Our results have indicated that the recruitment of naturally discharging cells is dependent, at least partly, on the membrane properties related to the size principle. Considerable overlap of these electrical parameters (Fig. 6) among cell types indicates that recruitment order does not accurately predict the membrane properties. After determining the $R_m$ in cats with hypocapnic apnea, Jodkowski et al. [3] elevated the level of CO$_2$ to restore rhythmic respiratory activity. Type H cells
Table 2. Comparisons of electrical properties of phrenic motoneurons among adult rat, newborn rat, and adult cat.

<table>
<thead>
<tr>
<th></th>
<th>Adult rat in vivo, 37°C</th>
<th>Newborn rat in vitro, 27°C</th>
<th>Adult cat in vivo, 37°C</th>
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<tr>
<td>E type (%)</td>
<td>23</td>
<td>28</td>
<td>28</td>
<td>[5]</td>
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<tr>
<td>L type (%)</td>
<td>42*</td>
<td>24</td>
<td>48</td>
<td>[5]</td>
</tr>
<tr>
<td>Q type (%)</td>
<td>34</td>
<td>48</td>
<td>48</td>
<td>[5]</td>
</tr>
<tr>
<td>$E_m$ (mv)</td>
<td>-67</td>
<td>-70</td>
<td>-69</td>
<td>[5,9]</td>
</tr>
<tr>
<td>AHP$_{dur}$ (ms)</td>
<td>55</td>
<td>75</td>
<td>75</td>
<td>[5]</td>
</tr>
<tr>
<td>APW$_{1/2}$ (ms)</td>
<td>0.51**</td>
<td>0.64</td>
<td>0.64</td>
<td>[5]</td>
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<tr>
<td>$R_m$ (MΩ)</td>
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<td>28</td>
<td>1.3</td>
<td>[3,9]</td>
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<tr>
<td>$I_{th}$ (nA)</td>
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<td>0.6</td>
<td>9.7</td>
<td>[3,9]</td>
</tr>
<tr>
<td>MPPI (ms)</td>
<td>1.9</td>
<td>2.2</td>
<td>2.2</td>
<td>[1]</td>
</tr>
</tbody>
</table>

Reference numbers give the sources of data. *Included both types L and VL cells. **This value was calculated from 51 cells having APW$_{1/2}$ less than 0.8 ms.

($R_m > 1.3$ MΩ) were recruited either in early or late stages of inspiration (the number of cells tested was only 4). No type L cells ($R_m < 1.3$ MΩ) fired, and they were considered as type Q cells. The present and previous studies have indicated that both the membrane properties and extrinsic factors such as the characteristics of synaptic inputs contribute to the recruitment order of spontaneously discharging PMs. The relative extent of their contributions remains to be determined [8, 12, 21].

The electrical properties of rat and cat PMs are compared in Table 2. No appreciable differences were noted between the rat and cat in the proportion of cell types and $E_m$. AHP$_{dur}$, $R_m$, $I_{th}$, and MPPI were different between the two species. The distribution of APW$_{1/2}$ was bimodal and the wider APW$_{1/2}$ (>0.8 ms, 39 cells) could be due to the entry of Ca$^{2+}$ during impalement [22]. The average value of the narrower APW$_{1/2}$ (<0.8 ms) was calculated to be 0.51 (±0.11 ms, SD, n = 51) and this value was narrower than that reported for cat PMs [5]. In spinal motoneurons, including PMs, AHP$_{dur}$ is a factor that limits the firing frequency [1, 3, 5]. The MPPI is a measure of the absolute refractory period and might also relate to the maximum firing frequency. APW$_{1/2}$ indicates the absolute refractory period. Therefore, the shorter AHP$_{dur}$, APW$_{1/2}$, and MPPI in rat than in cat PMs give rise to higher frequency responsiveness, as reflected partly in the spontaneous discharge rates (Table 2, mean $f$). A higher $R_m$ and a lower $I_{th}$ in the rat than in the cat might reflect the smaller size of rat PMs. The $R_m$ in the present study might have been underestimated because of the presence of IPSPs during expiration. Our previous study, using the intracellular labeling technique [20], showed that the short and long axes of rat PMs were smaller by approximately 20% than those of cat PMs analyzed in the same plane [23].

To calculate the axonal conduction velocity, the distance from stimulus sites on the thoracic phrenic nerve to the entry site of the anterior root into the spinal
cord at C₄ was measured in three animals (body weight, 380–420 g), and averaged as 86 mm. The mean AL-t was 2.0 ms (n = 15). If the time required to activate the axon is assumed to be 0.1 ms and if the intra-spinal axonal distance was 1 mm, the conduction velocity was approximately 46 m/s. This value is 22% slower than that measured in cat PMs (59 m/s [2]). It has been demonstrated that the axonal conduction velocity of fibers innervating the medial gastrocnemius is slower in rats than in cats [24].

Smith et al. [9] reported a much higher Rₘ and a much lower Iₘ measured from neonatal rat phrenic motoneurons than those determined in this study (Table 2). These differences could be attributed to developmental issues on the somal size, dendritic morphology, and synaptic connectivity [5] and to the in vitro condition at a lower temperature (27°C).

PMs were actively inhibited during phase II expiration (late expiratory period). The pattern of this inhibition was ramp-like with the progression of expiration. Using the spike-triggered averaging technique, Merrill and Fedorko [25] have reported that in the cat, expiratory neurons with an augmenting discharge pattern located in the Bötzinger area project to the spinal cord and inhibit PMs monosynaptically. It is likely that at least part of the IPSPs recorded in our study may originate from the respiratory neurons located in the Bötzinger areas. In the rat, Pilowsky et al. [6] have reported that expiratory neurons located in the rostral ventrolateral medulla project to the spinal cord. Segmental interneurons are another possible source of the inhibition. The presumptive expiratory interneurons recorded in our study showed CRDP of 5–16 mV during expiration, but its plateau-like shape was different from the time course of the reversed IPSPs. We have recorded no neurons with a brief depolarization during post-inspiratory periods. Expiratory interneurons discharging during the post-inspiratory period have been described in the vicinity of the phrenic nucleus in the cat [26]. Post-inspiratory activity in the whole phrenic discharge [14] could not have been detected in the anesthetized rat.

Even during hypocapnic apnea, synaptic noise was present in the membrane potential trajectory of both the expiratory interneurons and PMs. It is possible that these interneurons receive the tonic inputs from the medullary center even during hypocapnic apnea and in turn inhibit PMs.

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